Dynamics and Fate of Brominated Flame Retardants (BFRs) in Simulated Sludge Treatment

Von der Fakultät für Lebenswissenschaften

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Saptono Hadi
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List of Contents

List of Abbreviations

ABS	acrylonitrile butadiene styrene
AE	aerobic
AMAP	Arctic Monitoring and Assessment Program
AN	anaerobic
AN-AE	sequential anaerobic-aerobic
APCI	atmospheric pressure chemical ionization
APPI	atmospheric pressure photoionization
AU	absorption unit
BCF	bioconcentration factor
BDE-209	decabromodiphenyl ether
BFRs	brominated flame retardants
BPA	bisphenol A
BFRIP	Brominated Flame Retardant Industry Panel
BSEF	Bromine Science and Environmental Forum
CAS	Chemical Abstracts Service
CDT	cyclododecatriene
deBDethane	decabromodiphenyl ethane
deca-BDE	decabromodiphenyl ether (commercial mixture with BDE-209 as dominant congener)
CEPA	Canadian Environmental Protection Act
CFU	colony-forming unit
Da	Dalton
DCM	dichlormethane
DO	dissolved oxygen
d.s.	dry substance
DT_{50}	time required for 50% dissipation
EC	European Commission
ECHA	European Chemicals Agency
ECNI	electron capture negative ion
EDC	endocrine disruptor compound
EFRA	European Flame Retardants Association
EFSA	European Food Safety Authority
EI	electron impact
EMV	electron multiplier voltage

EPS	expanded polystyrene
ESI	electrospray ionization
EU	European Union
FID	flame ionization detector
f.w.	fresh weight
GC/MS	gas chromatography coupled with mass spectrometry
GPC	gel permeation chromatography
HBCD	hexabromocyclododecane
ΣHBCD	sum of α, β, and γ-hexabromocyclododecane
HIPS	high impact polystyrene
HPLC/DAD	high performance liquid chromatography coupled with diode array detector
HPVC	high production volume chemical
HRMS	high resolution mass spectrometry
i.d.	inner diameter
IPCS	International Programme on Chemical Safety
IUPAC	International Union of Pure and Applied Chemistry
P_{ow}	octanol-water partition coefficients
LC/MS/MS	liquid chromatography coupled with tandem mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
LPVC	low production volume chemical
LRMS	low resolution mass spectrometry
l.w.	lipid weight
MAE	microwave-assisted extraction
MAP	magnesium ammonium phosphate
MSPD	matrix solid-phase dispersion
m/z	mass to charge ratio
n.d.	not detected
NP	normal phase
octa-BDE	octabromodiphenyl ether
PBBs	polybrominated byphenyls
PBDD/Fs	polybrominated dibenzo-*p*-dioxins and furans
PBDEs	polybrominated diphenyl ethers
PBT	persistent, bioaccumulative, toxic
PCB	polychlorinated biphenyl

penta-BDE	pentabromodiphenyl ether
PLE	pressurised liquid extraction
POPs	persistent organic pollutants
PTV	programmable temperature vaporization
REACH	Registration, Evaluation, Authorization and Restriction of Chemical
RoHS	Restriction of Hazardous Substances
RP	reversed phase
RSD	relative standard deviation
SCOP	Stockholm Convention on POPs
SD	standard deviation
SECURE	Self-Enforced Control of Use to Reduce Emissions
S/N	signal to noise ratio
SPME	solid-phase microextraction
SRT	sludge retention time
SVHC	substances of very high concern
TBBPA	tetrabromobisphenol A
TCD	thermal conductivity detector
TKN	total Kjeldahl nitrogen
TOC	total organic carbon
t_R	retention time
TSCA	Toxic Substances Control Act
UAE	ultrasonic-assisted extraction
UBA	Umweltbundesamt
UNEP	United Nations Environmental Program
USEPA	United States Environmental Protection Agency
UV/Vis	ultraviolet/visible
VECAP	Voluntary Emissions Control Action Program
VFA	volatile fatty acid
vPvB	very persistent and very bioaccumulative
v/v	volume to volume ratio
WEEE	Waste of Electrical and Electronic Equipment
w/w	weight to weight ratio
WWTP	wastewater treatment plant
XPS	extruded polystyrene

1. Introduction

1.1 BFRs as emerging environmental pollutants

The increasing environmental contamination by numbers of industrial chemicals is one of the major environmental issues today. The use of chemicals in the human culture already started in the pre-industrial era. However, during the industrial revolution, a vast advance was made in invention and manufacturing processes. Thus, enormous numbers of new chemicals were invented and manufactured. The use of a wide range of industrial chemicals, e.g. pesticides, pharmaceuticals, plasticizer, dyes, surfactants, etc., since then has constantly increased up to recent years. It is estimated that around 100,000 new chemicals have been introduced into the environment during the 20th century (Bargagli, 2005). However, in contrast with their extensive usage, many of those synthetic chemicals are not routinely monitored and knowledge about their adverse effect on the environment and human health is still limited. They are thus classified as emerging contaminants that cover a broad range of substances, including nanoparticles, microbial contaminants, inorganic compounds, and a large part of organic chemicals (Balducci et al., 2012).

Brominated flame retardants (BFRs) are a group of compounds that have received much attention as “emerging” contaminants during the last years. BFRs are a group of flame retardants that were first commercialized in the 1950s. The market demand of flame retardants has been constantly growing, corresponding to the increasing usage of polymeric materials in construction, textiles, electronics, and computer equipments. Flame retardants were regularly added to polymers, which are mostly petroleum-based and thus are highly flammable, as an effort to reduce fire accidents. It was reported that the total direct and indirect cost of fire accidents was reached about 25 billion € per year in Europe (EFRA, 2007). As a result, flame retardants application in domestic and industrial products became a part of fire safety regulation. The world-wide current production of flame retardants was estimated to about 1.2 million t/y of which around 36% of the total market were brominated derivatives (Guerra et al., 2011). Thus, BFRs became one of the largest sales markets of industrial chemicals because of their performance and cost-effectiveness. In the meantime, the environmental safety of BFRs has become the major concern in terms of their fate, stability, and accumulation in the environment.

The interest of BFRs as emerging pollutants started in the 1970s as a result of the accidental feed contamination with a commercial BFR formulation of polybrominated biphenyls (PBBs), Firemaster FF-1, or known as “Michigan Incident”. During this incident, Firemaster FF-1 was

accidentally added to cattle feed instead of a dietary supplement magnesium oxide (Damstra et al., 1982). Signs of intoxication of cattle (decreased milk production, anorexia, alopecia, abnormal growth) were detected following the accident, and these PBB-contaminated dairy and poultry products were finally consumed by Michigan residents. In the early 1980's, Anderson and Blomqvist discovered a high level of polybrominated diphenyl ethers (PBDEs) in fish samples from the Swedish River Viskan, and this was the first report of environmental contamination with BFRs (Guerra et al., 2011). This pioneer study was immediately followed by numerous studies about BFR occurrence in different environmental compartments. Currently, elevated BFR levels were apparently measured in various environmental matrices (indoor and outdoor air, dust, soil, sediment, sludge) and wildlife (de Wit, 2002; Hale et al., 2003; Alaee, 2006; Tanabe et al., 2007; Law et al., 2008; de Boer, 2009). Recent surveys also found deposits of BFRs in remote areas, such as the polar region, indicating long-range atmospheric transport of the chemicals (de Wit et al., 2006, 2010; AMAP, 2007; Meyer et al., 2012). In parallel, occurrence and increasing temporal trend of BFRs in human samples (breast milk, blood serum, scalp hair, etc.) have been discovered (Meironyte et al., 1999; Fängström et al., 2008; Eljarrat et al., 2009; Abdallah and Harrad, 2011; Malarvannan et al., 2013), revealing BFRs transfer from environment to human beings. Evidences of human exposure to BFRs from various routes were reported by several authors (Darnerud et al., 2001; Sjödin et al., 2003; Hites, 2004; Thuresson et al., 2006; Athanasiadou et al., 2008; Frederiksen et al., 2009). Those findings thus raised the concern on possible adverse effects by BFRs to human health. Toxic effects on human were reported, such as neurotoxic (Eriksson et al., 2001; Costa and Giordano, 2011) and endocrine disrupting effects (Zhou et al., 2001; Legler and Brouwer, 2003; Vos et al., 2003). BFRs were also reported to form polybrominated dibenzo-p-dioxins and furans (PBDD/Fs) during combustion (Wichmann et al., 2002; Ebert and Bahadir, 2003).

Sewage sludges as product from WWTPs (wastewater treatment plants) are widely recognized as an important sink of lipophilic and persistent organic contaminants (Allchin et al., 1999; Rayne et al., 2003). The discovery of BFRs in the environment was initially based on the analyses of sewage sludges, as well as sediments from an estuary impacted by the BFRs industry (Kierkegaard et al., 2004). In the late 1970s, the presence of BFRs in the effluent of industrial plants was reported for the first time by Di Carlo, in areas around PBDEs manufacturing facilities in Arkansas (USA) (Hale et al., 2006). Following this discovery, initial studies in Swedish WWTPs identified the presence of BFR residues, as PBDE congeners (Nylund et al., 1992) and subsequently non-PBDE BFRs (Sellström and Jansson, 1995; Öberg et al., 2002). At the same time, the occurrence of BFRs in effluents and sewage sludges of WWTPs was also measured in other European countries (de Boer et al., 2000;

Christensen et al., 2003; Law et al., 2006a; Knoth et al., 2007; Díaz-Cruz et al., 2009; Cincinelli et al., 2012, Zennegg et al., 2013), and more recently in other countries worldwide (Wang et al., 2007; Clarke et al., 2008; Gevao et al., 2008; Hwang et al., 2012; Daso et al., 2012; Ilyas et al., 2013). Those findings indicated sewage sludge as the primary sink for BFRs. Therefore, sewage sludge can be an important source for environmental contamination by BFRs, for example, when it is applied to agricultural soil as fertilizer. It is estimated that more than 11 million t/y of dry sewage sludge is generated in the EU countries (data predicted for 2010; de la Torre et al., 2011), for which the agricultural application is one of the predominant disposal routes. The proportion varies between the EU countries, which the highest proportion was reported for Spain (around 65%) while in other countries, i.e. Finland and Greece, the proportion was less than 5% (de la Torre et al., 2011).

Before being used for agricultural application, the raw sludge is normally further treated in order to meet the regulatory standards. European regulations established limit values for contaminants in sludge for agricultural application, such as Directive 86/278/EEC of 12 June 1986 for selected heavy metals. Limit values for organic compounds, i.e. dioxin and PCBs, are also defined in several national regulations, such as Germany (AbfKlärV, 2012). However, an equivalent regulation for BFRs has so far not been established (Sellström et al., 2005). The recent abundance of BFRs residues in sludge reflects an extensive use of these compounds. The fate of organic compounds in WWTPs is basically determined by their physicochemical properties, the process design, and operating conditions at the treatment system (Katsoyiannis and Samara, 2004). Many recent studies reported the different performance of conventional WWTPs in elimination for different classes of organic compounds (Kujawa-Roeleveld et al., 2006; Kosjek et al., 2007; Jelíc et al., 2012). As a highly hydrophobic compound, an efficient removal rate of BFRs has been achieved in WWTPs effluent. Ricklund et al. (2008a) reported that less than 1% of deca-BDE is present in effluent, and it was almost completely enriched in sludge. Thus, inadequate elimination during subsequent sludge treatments is perhaps the main reason for the continuous emission of those compounds to the environment. Information on BFRs fate in sludge, such as their resistance to degradation, is currently still limited.

1.2 Mode of action of BFRs

Flame retardants act by an interference of particular step of a combustion process. Combustion is a gas-phase self-sustaining reaction, involving combustible material, heat, and oxygen (from air or oxidants), that can be divided into four main phases: (1) preheating, (2) decomposition, (3) combustion, and (4) propagation (Troitzsch, 1998) (**Fig. 1.1**). The

initial step of combustion involves an endothermic heating of the combustible material by a sufficient external source (preheating phase). This results in thermal decomposition of the materials (pyrolysis phase), which is accompanied by a release of flammable gases. As the next step, the gases will react with oxygen from the air. This reaction is an exothermic process and produces visible flames (combustion phase). As the final step, an excess of heat is generated allowing a thermal feedback. In this stage, the burning process becomes self-propagating (propagation phase), and the material will continuously decompose.

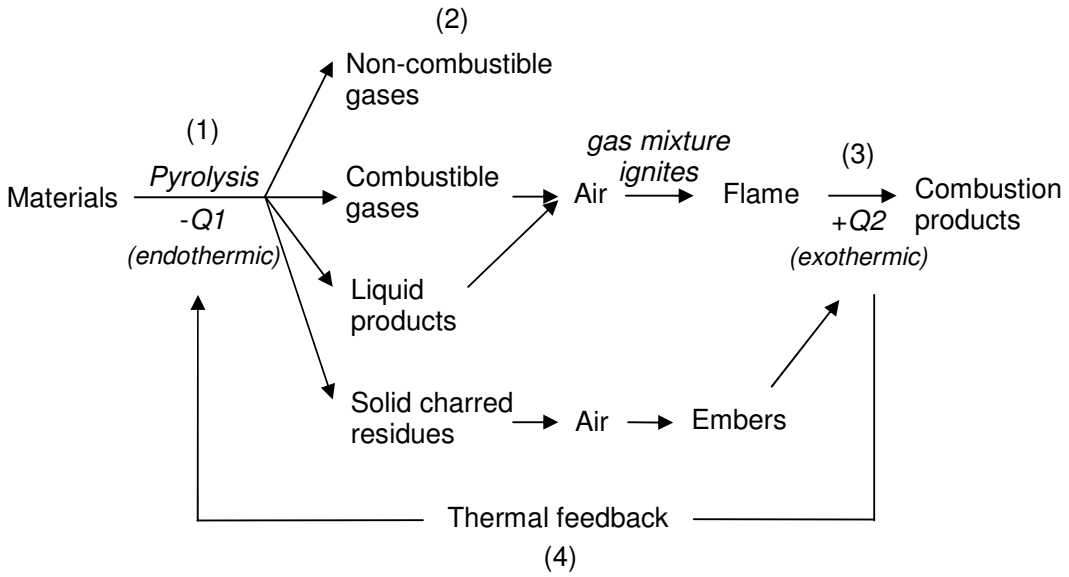


Figure 1.1: The main steps of the combustion process: (1) preheating, (2) decomposition, (3) combustion, and (4) propagation (Troitzsch, 1998)

BFRs mainly act through a chemical suppression of the radical chain reaction that takes place during the decomposition phase of the combustion process. Free radicals are widely known as an important element for the flame propagation. At high temperatures, BFRs will release $Br^{\bullet}$ radicals, which react with the hydrocarbon molecules of flammable gases to produce HBr. Based on the radical trap theory of flame inhibition (Green, 1996), the produced HBr will subsequently react with the radical species $^{\bullet}OH$ and $H^{\bullet}$, forming H_2O, H_2, and $Br^{\bullet}$ radicals (Eq. 1.1). The reactive $^{\bullet}OH$ and $H^{\bullet}$ will be substitute by less reactive $Br^{\bullet}$ radicals, which are essential for the proliferation of flame. Thus the propagation process is finally terminated, resulting in flame extinguishment.

$$\text{R-Br} \rightarrow \text{R}^{\bullet} + \text{Br}^{\bullet} \qquad (1.1)$$
$$\text{Br}^{\bullet} + \text{R-H} \rightarrow \text{R}^{\bullet} + \text{HBr}$$

$$HBr + OH^{\bullet} \rightarrow H_2O + Br^{\bullet}$$
$$HBr + H^{\bullet} \rightarrow H_2 + Br^{\bullet}$$

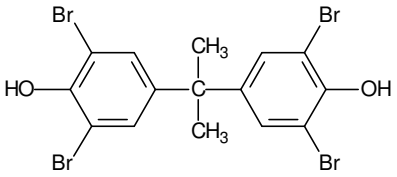
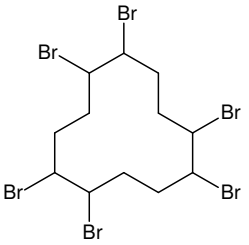
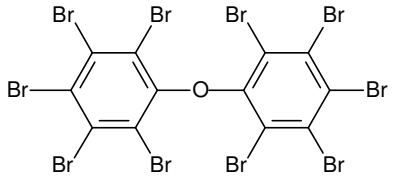
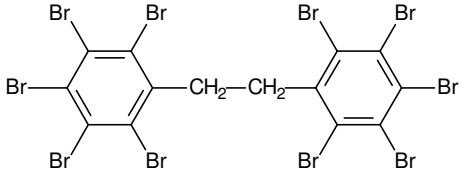
All halogen compounds are able to eliminate free radicals during combustion reactions. However, the trapping efficiency of halogens is corresponding to their atomic size. Therefore, their performance as flame retardant increases as follow: I > Br > Cl > F (Green, 1996). Considering to higher trapping efficiency and also moderate decomposition temperature, bromine-based compounds are preferable over chlorine-based compounds (Guerra et al., 2011). Iodine-based compounds, although effective, are not commercially used because either chemically unstable and expensive.

1.3 BFRs compounds under study

BFRs cover more than 75 different chemicals with a broad range of physicochemical properties (Birnbaum and Staskal, 2004). Therefore, their environmental fate and behaviour can be very different. From these 75 different chemicals, 4 test compounds were selected for this study, as summarized in **Tab. 1.1**.

The selection of the test compounds was primarily based on the importance of the compounds, which corresponds to their current production and commercial usage (**Ch. 1.3.2**, **Tab. 1.2**). The selected compounds should also cover the wide range of physicochemical properties which is varying among different groups of BFRs. In general, BFRs have a low vapour pressure (V_p), low solubility in water (S_w), and high lipophilicity due to their high octanol/water partition coefficient (log P_{ow}). BFRs can be divided into three major chemical classes: (1) aromatics, e.g. tetrabromobisphenol A (TBBPA) and PBDEs; (2) cycloaliphatics, e.g. hexabromocyclododecane (HBCD); and (3) aliphatics, e.g. dibromoneopentyl glycol. The third represents a minor group of substances. Based on their mode of incorporation into polymeric material, BFRs can be further divided into two classes, reactive and additive BFRs. Reactive BFRs, such as TBBPA, are added before polymerization and covalently bound to the polymer. In contrast, additive BFRs, such as PBDEs and HBCD, are just mixed with the other components of the polymers and are not chemically incorporated in the polymer matrices. Beside those emerging BFRs, a novel BFR compound, decabromodiphenyl ethane (deBDethane), was also included to the test compounds, considering the scarcity of environmental data and also its importance as replacement compound, particularly for deca-BDE. DeBDethane is recently introduced to the market (Betts, 2008; Covaci et al., 2011a) and regarded as the successor for deca-BDE due to their structural resemblance (Kierkegaard, 2007).

Table 1.1: Chemical identity of selected BFRs as test compounds

Chemical Name/CAS No.	MW [g/mol]	Chemical structure	M_p [°C]	V_p [Pa]	S_W [µg/L]	Log P_{ow}
Tetrabrobomisphenol A CAS No.: 79-94-7	543.9		181-182[a]	6.2×10^{-6} [a]	240[a]	5.90[a]
Hexabromocyclododecane CAS No.: 25637-99-4 (technical)	641.7		~190[b]	6.3×10^{-5} [b]	65.6[b]	5.63[b]
Decabromodiphenyl ether CAS No.: 1163-19-5	959.2		300-310[c]	4.6×10^{-6} [c]	<0.1[c]	6.27[c]
Decabromodiphenyl ethane CAS No.: 84852-53-9	971.2		348-353[d]	$<1\times10^{-6}$ [d]	0.72[d]	>7[d] (calculated)

MW = molecular weight; M_p = melting point; V_p = vapour pressure; S_W = water solubility; Log P_{ow} = log of the octanol-water partition coefficients

[a]ECHA, 2006; [b]ECHA, 2008a; [c]ECHA, 2002; [d]Dungey and Akintoye, 2007

1.3.1 Properties of the selected BFRs

TBBPA structurally consists of two hydroxyphenyl rings linked by a carbon bridge with four bromines substituted at the 3, 3', 5, and 5' position (**Tab. 1.1**). TBBPA is manufactured by bromination of bisphenol A (BPA) in the presence of solvent, such as methylene chloride. The technical product of TBBPA generally has a high degree of purity (>98.5%) (IPCS, 1995), and impurities consist of lower brominated TBBPA congeners, mainly tribromobisphenol A (ECHA, 2006). TBBPA manufacturing by-products, PBDD/Fs, may also be present at trace levels (ECHA, 2006). Subsequent formation of highly toxic PBDD/Fs is also possible during TBBPA processing, application, accidental fires, and disposal (e.g. incineration) of TBBPA-containing products (Ebert and Bahadir, 2003).

TBBPA is a white crystalline or white powdered solid compound, which has a bromine content of approximately 58% w/w. TBBPA has a low vapour pressure, low solubility in water, and high lipophilicity due to its high octanol/water partition coefficient (log P_{ow}) value. As a phenolic compound, TBBPA is a weak acid and thus can exist in the form of undissociated (neutral) or dissociated (ionized) species. TBBPA has two acidic hydrogen atoms, thus also two pK_a values (IPCS, 1995).

HBCD is a non-aromatic BFR, which consists of a cycloaliphatic ring of 12 carbon atoms to which six bromine atoms are attached. It is produced by bromination of *cis–trans–trans*-1,5,9-cyclododecatriene (CDT) in a batch process which results in the formation of theoretically possible 16 stereoisomers, including 6 enantiomeric pairs and 4 meso forms (Law et al., 2005). Other trace impurities are also possibly present in the reaction product, such as tetrabromocyclododecene (ECHA, 2008a). The commercial product of HBCD [CAS 25637-99-4] is generally designated as non-specific mixture of all isomers, which is predominantly constituted by three main diastereoisomers α-, β-, and γ-isomer (Tomy et al., 2005) (**Fig. 1.2**). The isomer composition in the technical product is dominated by γ-isomer, contributing to approximately 80% of formulation, and is followed by α- (12%) and β-isomer (6%) (Heeb et al., 2005).

Technical HBCD is a white solid substance that has 75% w/w bromine content. HBCD has similar physicochemical characteristics to other BFRs, such as low volatility, high lipophilicity, and low solubility in water. However, HBCD is a distinctly thermolabile compound, and thermal rearrangement of isomers occurs at elevated temperature (Barontini et al., 2001). Differences in the structure of the polymers cause disparities in their physicochemical properties, such as the log P_{ow} and water solubility (ECHA, 2008a).

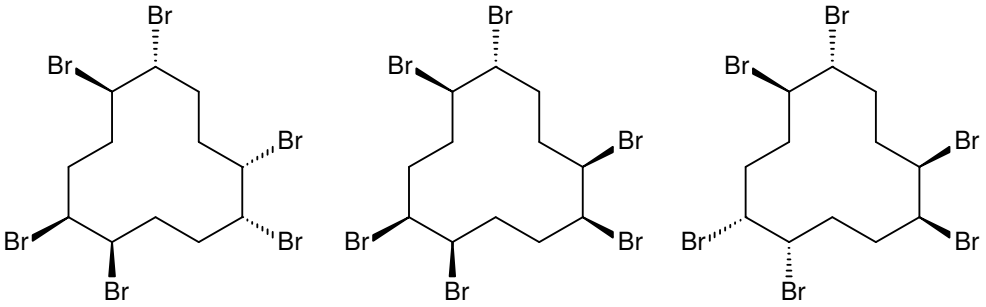


Figure 1.2: Chemical structure of the three main stereoisomers of HBCD (α, β, and γ) which dominate in technical products

Deca-BDE is one congener of the PBDEs, which have the general chemical formula $C_{12}H_{(9-0)}Br_{(1-10)}O$. Structurally, PBDEs are characterized by an ether-bridged aromatic structure (**Tab. 1.1**) and consist of 209 theoretically possible congeners depending on the number and position of the bromine atoms on the phenyl rings. PBDEs nomenclature is designated by numbers, which is proposed by Ballschmiter and Zell and refers to PCB nomenclature system (Bergman et al., 2012). As a group of compounds, PBDEs are divided into 10 congener groups (mono- to deca-BDE). Three mixtures of penta, octa, and deca congeners are available as commercial products. Deca-BDE is a fully brominated PBDE congener, which consists of 10 bromine atoms attached to the aromatic rings and has a bromine content of 83% w/w. PBDEs are produced by direct bromination of diphenyl ether using a Friedel-Crafts catalyst. The technical product of deca-BDE is predominantly composed of BDE-209 (97-98%) and a small amount of nona-BDEs (0.3-3%) (ECHA, 2002). The presence of PBDD/Fs as impurities has been recently reported (Ren et al., 2011).

Technical deca-BDE is a fine, white to off-white crystalline powder. Deca-BDE has a low vapour pressure at room temperature and decomposes at 320 °C. The solubility of deca-BDE is extremely low in water and also very limited in common organic solvents. Solubility of deca-BDE in various organic solvents is as follow: 0.05% in acetone, 0.48% in benzene, 0.42% in methylene bromide, 0.87% in xylene, and ~0.2% in toluene (ECHA, 2002). Deca-BDE is a high lipophilic substance with high log P_{ow} value of 6.27. BDE-209 is commonly used as a synonym for technical deca-BDE, since BDE-209 is a dominant congener in the technical product.

DeBDethane has a structural similarity to deca-BDE only the ether bridge between the two aromatic rings in deca-BDE is replaced by an ethane bridge in deBDethane (**Tab. 1.1**).

Structurally, the ethane bridge between the aromatic rings introduces more conformational flexibility in the deBDethane molecule, but it also produces lower polarity, which means that the ethane is more hydrophobic than the ether (Dungey and Akintoye, 2007). In contrast to deca-BDE, deBDethane does not have an ether bridge in its molecular structure, thus has less possibility to produce PBDD/Fs under pyrolysis condition (Kierkegaard et al., 2004). Technical deBDethane has a typical purity of ≥98.5% (Dungey and Akintoye, 2007). Lower brominated congeners (mainly of nonabromodiphenyl ethane) are generally present in deBDethane as impurities (Covaci et al., 2011a). Bromination of the ethane bridge, producing "over-brominated" species, is also possible. These molecules are found at trace levels (Dungey and Akintoye, 2007).

Technical deBDethane is an odourless white or off-white powder, which has approximately 82% w/w bromine content (Albemarle, 2001). Based on the structural resemblance of deBDethane to deca-BDE, their physicochemical properties are similar, e.g. low volatility, low water solubility, and high log P_{ow} (Hardy et al., 2002). Solubility in organic solvents (acetone, methanol, hexane, toluene, chlorobenzene, methyl ethyl ketone, methylene dibromide, dimethyl formamide) is also extremely low (<0.01% at 25 °C) (Albemarle, 2001).

1.3.2 Production, consumption, and application

Production and consumption

In Europe, TBBPA, HBCD, and deca-BDE are classified as high production volume chemicals (HPVC), as their market volume exceeds 1,000 t/y. Among them, TBBPA has the largest production volume, representing up to 60% of the global BFR market in 2001 (**Tab. 1.2**).

Table 1.2: Estimated market demand (t) of BFRs by region in 2001

Compounds	Europe	America	Asia	The Rest	Total
TBBPA[a]	11,600	18,000	89,400	600	119,600
HBCD[a]	9,500	2,800	3,900	500	16,700
deca-BDE[a]	7,600	24,500	23,000	1,050	56,150
deBDethane[b]	2,500	–	–	–	–

[a]BSEF, 2003; [b]Dungey and Akintoye, 2007; – = current data are not available

TBBPA is produced as a primary product, in partially finished products (e.g., polymers and epoxy resins), or finished products (e.g., electronic equipments) (Guerra et al., 2011). TBBPA consumption in the EU was estimated ≥11,000 t/y, mostly imported in finished products (ECHA, 2006). HBCD is the main aliphatic cyclic additive BFRs in the market today and is also the third most used BFR, followed by TBBPA and PBDEs. HBCD is extensively used in the EU. Its consumption reached 9,500 t/y (BSEF, 2003). Deca-BDE represents the major product of PBDE mixtures and the second largest market (after TBBPA), which is estimated to reach 56,100 t/y (BSEF, 2003). In Europe, deca-BDE consumption rate is comparatively lower than in North America. Its consumption peaked in the late 1990s at approximately 9,000 t/y and declined by 30% in 2010 (Earnshaw et al., 2013). Information on recent production and market volume of deBDethane are still limited. In Europe, deBDethane is classified as a low production volume chemical (LPVC) or ≤1,000 t/y. DeBDethane is not manufactured in Europe. However, import of the deBDethane Saytex 8010 to Europe, mainly Germany, was estimated to be 2,500 t in 2001 (Dungey and Akintoye, 2007). One reason for the increasing usage of deBDethane was that it fulfills the German dioxin ordinance, which regulates limits for dioxins and furans in commercial products (Kierkegaard et al., 2004). During 1993 to 2000, the global use of deBDethane was constantly increased whereas deca-BDE consumption decreased during the same period (Kierkegaard et al., 2009).

Application

BFRs are used in order to reduce fire risks in many kind of materials that are susceptible to burning (plastics, wood, textiles, etc.). Therefore, their application pattern depends on many factors, such as its suitability for the material that shall be protected and the fire safety standards that have to be fulfilled.

TBBPA is primarily used as a reactive flame retardant in various polymers, mainly epoxy, vinyl esters, and polycarbonate resins. The main application of TBBPA is in epoxy resins for electronic circuit boards assembly and encapsulation of electronic components. In the resin, the phenolic hydroxyl group of TBBPA will covalently react with the polymer backbone and become an integral part of the polymer structure. A minor portion of TBBPA is also used as an additive flame retardant in polymers, such as acrylonitrile butadiene styrene (ABS), and high impact polystyrene (HIPS), which is only accounted for about 20% of the total consumption (ECHA, 2006). TBBPA is also used as a parent compound for the production of TBBPA derivatives, other BFR group of compounds, and brominated epoxy oligomers (BSEF, 2013a).

HBCD is an additive flame retardant which is mainly used in thermoplastic polymers, primarily in styrene resins, including expanded polystyrene (EPS) and extruded polystyrene (XPS) (BSEF, 2013b). Those polymers are commonly used in building and construction materials, such as for thermal insulation. To a lesser extent, HBCD has been used in combination with a synergist antimony trioxide in back-coatings of textiles, mostly for upholstered car seatings, upholstered furnitures, draperies, wall coverings, etc. (Morose, 2006). In Europe, <10% of HBCD has also been applied in HIPS, a rigid thermoplastic material mostly used in electrical and electronic appliances (ECHA, 2008a). Application of HBCD is beneficial due to its effectiveness at low concentrations.

Deca-BDE is another additive flame retardant that has a general purpose due to its effectivity and versatility. In plastics, deca-BDE is used for electrical and electronic equipments (e.g. computers, connectors, electrical boxes), transportation (e.g. cars and aviation industries), and construction and building materials (e.g. wires, cables, pipes). While in textiles, deca-BDE is used in upholstered furniture, draperies, and synthetic carpets (BSEF, 2013c). However, the major application of deca-BDE is in HIPS, which is widely used for TV sets and computers cabinets (ECHA, 2002).

As a commercial product, deBDethane was introduced in the early 1990s under the trade name Saytex 8010 by Albemarle Corporation (Kierkegaard et al., 2004). It is currently an important alternative to deca-BDE with its applications very similar to those of deca-BDE. DeBDethane is used together with antimony trioxide in the ratio 2:1 to 3:1 (UBA, 2001), and applied as an additive flame retardant in variety of polymeric materials, i.e. in textile back-coatings (Dungey and Akintoye, 2007).

1.3.3 Sources, transport, and occurrence

Sources

Possible emission of BFRs to the environment occurs during life cycle of BFRs-containing materials. Production, application, and disposal can be a point or diffuse emission sources (Remberger et al., 2004). Reactive BFRs, such as TBBPA, are relatively stably incorporated into the respective materials and are not easily released from the final product (Covaci et al., 2009). Therefore, the potential for environmental emission of TBBPA particularly comes from the excess of the compound which is added to the treated materials during a manufacturing process (Covaci et al., 2009). In contrast, additive BFRs such as HBCD and PBDEs, have a higher possibility to be released into the environment during their application. That may occur by abrasion (particulates) or direct leaching from their polymer host (Alaee et al., 2003). It

was also shown that additive chemicals are mainly emitted from polymers by diffuse processes (Herrmann et al., 2002; Cox et al., 2002). During product application, BFRs may be emitted to air. However, due to their low volatility, emission by particulate is probably a more relevant route than direct volatilization (Liagkouridis et al., 2014). Release of BFRs via plant effluents is expected to be limited since the industrial processes are conducted in a closed system (Remberger et al., 2004). Release from incineration plants is also not considered as a major source as BFRs are degraded at operating temperatures of incinerators. However, trace amounts could be released from the combustion chamber (UNEP, 2006). Disposal sites, including recycling plants and landfills, are also possible emission sources, particularly to soil, surface waters, and groundwater. Industrialised or urban areas are expected as substantial BFR sources, corresponding to higher population density and commercial products consumption (Hale et al., 2006; Ilyas et al., 2011).

Transport

BFRs can be transported to WWTPs via atmospheric deposition, urban run-off, and the contribution of domestic and industrial discharges that are collected through the sewerage system (**Fig. 1.3**).

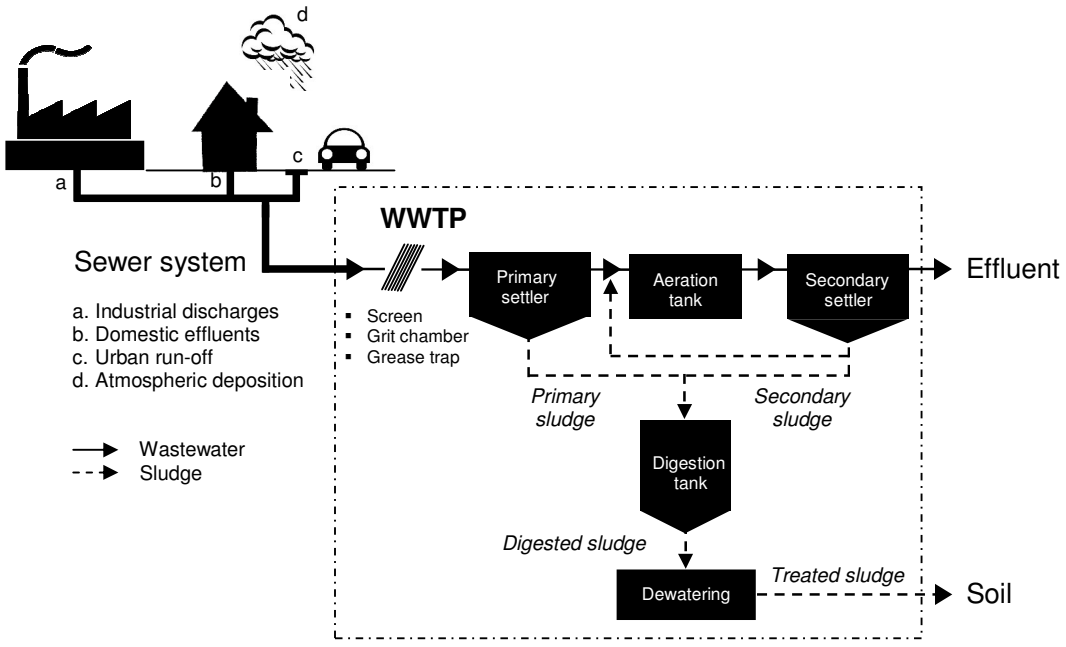


Figure 1.3: Principle pathways of BFRs to the environment via WWTP and sludge application on soil

During steps of treatment in WWTPs, due to their extremely hydrophobic character, BFRs are preferably associated with the solid fractions, thus resulting in accumulation in sewage

sludge (Hale, 2002; North, 2004; Langford et al., 2005). Furthermore, high removal efficiency of solid organic matters in WWTPs has a direct correlation with an effective elimination of the hydrophobic contaminant from WWTPs effluents. With respect to the environmental behavior of BFRs, increasing bromination will decrease water solubility and increase P_{ow}. Mass balance studies of higher-brominated BFRs demonstrated an effective removal of BFRs by solids settling during sewage treatment process. Song et al. (2006) showed that only a small fraction (<10%) of the Σpenta-BDE mass will be discharged via the final effluent, whereas a significantly higher removal (<1% in the effluent) was reported by Ricklund et al. (2008a) for deca-BDE and deBDethane. Therefore, sewage sludge represents a relevant route for BFRs release from the technosphere to the environment.

Occurrence

BFRs were found at different concentrations in all relevant environmental compartments. From previous studies, BFRs were detected in indoor air (Tollbäck et al., 2006; Karlsson et al., 2007; Abdallah et al., 2008a, 2008b; Takigami et al., 2009) and indoor dust (Leonards et al., 2001; Takigami et al., 2009; Ali et al., 2011; Kang et al., 2011; Kopp et al., 2012; Coakley et al., 2013). BFRs levels in indoor air (i.e. home, office, cars) were detected at levels of ng/m^3 and found higher (up to 5 orders) compared to outdoor air (Harrad et al., 2008, 2010), indicating BFRs emission from household materials and textiles during weathering of those products. The detected levels in the rural outdoor (Xie et al., 2007) and remote areas, such as the Arctic (AMAP, 2005; de Wit et al., 2006, 2010; Meyer et al., 2012), imply that BFRs have the potential for long-range transport. BFRs were found also in other abiotic compartments, such as water (Remberger et al., 2004; Suzuki and Hasegawa, 2006), leachates of waste disposals (Osako et al., 2004, Morris et al., 2004), soil (Sánchez-Brunete et al., 2009; Shi et al., 2009; Jiang et al., 2010; Xu et al., 2012; Zheng et al., 2012; Eguchi et al., 2013; Parolini et al., 2013), and sediments (Kierkegaard et al., 2004; Schlabach et al., 2004; Evenset et al., 2007; Ricklund et al., 2010; Klosterhaus et al., 2012; Moon et al., 2012). Levels measured in soil and sediment, at mg/kg d.s. suggested that those matrices are another major sink for BFRs.

BFRs were also measured in wildlife, including fish, birds, and marine mammals, etc. (Lindberg et al., 2004; Isobe et al., 2007; Kuiper et al., 2007; Johnson-Restrepo et al., 2008; Gao et al., 2009; Gauthier et al., 2009; Rüdel et al., 2012; Shaw et al., 2012). Furthermore, higher tropic level organisms, such as marine mammals and predator birds, showed higher levels of BFRs. Isobe et al. (2007) reported HBCD at a concentration range of 4.7-380 µg/kg (l.w.) in small cetaceans from the South China Sea. Janák et al. (2008) reported HBCD concentrations in some wildlife species from Sweden, which were found lowest in herring

(10s of µg/kg l.w.) and highest in top predator birds (100-1000s of µg/kg l.w.). These data indicate a biomagnification potential of BFRs across the food web. These data indicate a biomagnification potential of BFRs across the food web. BFRs were present in a number of foodstuffs, notably meat, fish, eggs, and dairy products (Gómara et al., 2006; Tlustos et al., 2007; Schecter et al., 2008; Päpke et al., 2010), thus may account for BFRs exposure to populations. In many recent studies, BFRs occurrences (at µg/kg l.w.) were reported also in human samples, such as blood (Jakobsson et al., 2002; Thomsen et al., 2007), adipose tissue (Antignac et al., 2009), breast milk (Meironyte et al., 1999; Fängström et al., 2008; Eljarrat et al., 2009; Carignan et al., 2012), and scalp hair (Zheng et al., 2011; Malarvannan et al., 2013), particularly in subjects who are occupationally exposed (i.e. workers at production, recycling, or disposal of BFR-containing products) and in the common populations.

Higher levels of BFRs were found in areas suspected of high exposure, such as ambient air close to BFRs manufacturing site (Zweidinger et al., 1979) and electronics recycling plant (Sjödin et al., 2001), soil and sediment samples from an estuary impacted by the BFRs industry (Kierkegaard et al., 2004; Remberger et al., 2004). Widespread occurrences in sewage sludge have been also reported in many studies, from low µg/kg d.s. levels up to high levels of >10,000 µg/kg d.s. in sludge samples taken from different WWTPs in Europe (**Tab. 1.3**).

Table 1.3: BFRs concentrations in sludge (µg/kg d.s.) from different WWTPs in Europe

Compound	Location	Conc. range [µg/kg d.s.]	References
TBBPA	Germany	0.6 - 62	Kuch et al., 2005
	Ireland	<2.4 - 192	Morris et al., 2004
	Netherlands	2 - 600	Morris et al., 2004
	Spain	<3 - 1,329	Guerra et al., 2010; Gorga et al., 2013
	Sweden	<0.3 - 220	de Wit, 2002; Öberg et al., 2002; Law et al., 2006a
	UK	15.9 - 112	Morris et al., 2004
HBCD	Ireland	153 - 9,120	Morris et al., 2004
	Netherlands	<0.6 - 1,300	Morris et al., 2004
	Spain	n.d. - 1,873	García-Valcárcel and Tadeo, 2009; Guerra et al., 2010

Table 1.3: Continued

Compound	Location	Conc. range [µg/kg d.s.]	References
	Sweden	<1 - 650	de Wit, 2002; Remberger et al., 2004; Law et al., 2006a
	Switzerland	39 - 597	Kupper et al., 2008
	UK	531 - 2,683	Morris et al., 2004
deca-BDE	Czech	5 - 320	Pulkrabová et al., 2007; Ricklund et al., 2008a
	Germany	6.4 - 2,217	Ricklund et al., 2008a; Hamm, 2004; Knoth et al., 2007
	Italy	130 - 9,411	Cincinelli et al., 2012
	Netherlands	1 - 330	de Boer et al., 2003
	Spain	<0.2 - 4,150	Martínez et al., 2006; Eljarrat et al., 2005; Gorga et al., 2013;
	Sweden	<0.6 - 1,100	Öberg et al., 2002; Law et al., 2006a; Ricklund et al., 2008b
	Switzerland	138 - 617	Kupper et al., 2008
	UK	(12,000)[a]	Ricklund et al., 2008a
deBDethane	Czech	6 - 140	Ricklund et al., 2008a
	Germany	<0.6 - 220	Ricklund et al., 2008a
	Spain	0.2 - 257	Eljarrat et al., 2005; Gorga et al., 2013; De la Torre et al., 2012
	Sweden	66 - 100	Kierkegaard et al., 2004; Ricklund et al., 2008b
	Switzerland	73 - 160	Ricklund et al., 2008a
	UK	34 - 63	Ricklund et al., 2008a

[a]The number in bracket refers to an extremely high concentration obtained from a single measurement.

1.3.4 Persistence

Persistence, based on EPA's definition, is the ability of a chemical to remain in an environment in an unchanged form. In the environmental context, persistence can be regarded as the residence time of a substance in a certain environmental compartment (i.e.

soil, sediment, water) (**Tab. 1.4**). Thus, persistence is a parameter, which is dependent on the chemical distribution among environmental compartments, as well as its degradation in each compartment.

Table 1.4: Persistence criteria (half-live, t½) for POP, PBT, and vPvB compounds

Compartment	**POP**[a,1]	**PBT**[b,2]	**vPvB**[b,3]
Soil	>180 d	>120 d	>180 d
Sediment	>180 d	>180 d (marine)	>180 d (marine)
		>120 d (fresh water)	>180 d (fresh water)
Water	>60 d	>60 d (marine)	>60 d (marine)
		>40 d (fresh water)	>60 d (fresh water)

[a]According to POPs protocol (UNEP, 2001), [b]According to REACH legislation (EU, 2006) [1]POP = persistent organic pollutants; [2]PBT = persistent, bioaccumulative, and toxic compounds; [3]vPvB = very persistent and very bioaccumulative compounds

BFRs, as other halogenated organic compounds, are considered as persistent compounds in the environment (Segev et al., 2009). It is indicated by increasing evidence of their occurrence in the environment at various locations, far away from production sites, and have even detected in remote areas (i.e. the Arctic). However, in certain environmental conditions, BFRs can undergo a variety of processes, which can affect their persistence. Degradation is an important process, which can contribute to the partial or complete elimination of the chemical and the formation of degradation products. Abiotic degradation, which involves chemical processes, mainly occurs in soil, water, and atmosphere via photolysis by sunlight. Other basic reactions, such as oxidation, reduction, hydrolysis–substitution–elimination (hse), and reactions with radicals are also possible mechanisms (Green and Bergman, 2005). Biodegradation, which involves biological processes, commonly takes place in soil or sediment compartment, which are largely populated by microorganisms.

Abiotic degradation

Based on a fugacity model, BFRs tend to partition to soil, sediment, or sludge following their release to the environment (Palm et al., 2002), where they may undergo degradation. In soil, abiotic oxidation reactions could have a significant role in the top layer of soil, while reduction is probably more relevant in water-logged soil or sediment (Nyholm, 2009). Laboratory studies investigating the stability to abiotic oxidation found that TBBPA is susceptible to oxidation with $KMnO_4$ (Bastos et al., 2008). Birnessite (δ-MnO2) mediated debromination

was investigated for TBBPA (Lin et al., 2009) and deca-BDE (Ahn et al., 2006), indicating oxidative degradation by naturally occurring metal oxides in soils and sediments as possible process. Reductive degradation of deca-BDE by zerovalent iron as a reducing agent has been also reported (Keum and Li, 2005; Zhuang et al., 2010), showing a correlation between the degree of bromination and the debromination rate. Hydrolysis is likely to be an insignificant route of environmental degradation for BFRs due to their very low water solubility (ECHA, 2002; 2008a; Dungey and Akintoye, 2007).

Photodegradation is considered as the most important mechanism for the abiotic degradation of BFRs, particularly for PBDEs. From laboratory studies, photolytic degradation of higher brominated PBDEs under different light sources were observed in organic solvents, such as methanol, hexane, tetrahydrofuran (THF), and toluene (Watanabe and Tatsukawa 1987; Bezares-Cruz et al., 2004; Eriksson et al., 2004a; Hagberg et al., 2006), as well as in solutions mixture, such as methanol/water (Eriksson et al., 2004a). PBDEs were also photolytically degraded in the variety of environmental matrices, such as sand and soil (Söderström et al., 2004), sediment and clay minerals (Ahn et al., 2006b), and house dust (Stapleton and Dodder, 2008). The degradation rates of PBDEs are slower in the natural matrices compared to those in the artificial materials. The photolytic degradation of deBDethane in a variety of organic solvent and matrices under artificial or natural sunlight irradiation has been reported (Wang, et al., 2012). Formation of lower brominated congeners (nona- and octa-BDethane) was identified during irradiation of the deBDethane solution under UV lamp (Kierkegaard, 2007; Wang, et al., 2010), indicating UV-mediated degradation of the compound. However, in the complex matrices, deBDethane is found to be more resistant. For example, no significant loss of deBDethane incorporated into HIPs polymer from TV casing was observed during 224 d of exposure to natural sunlight (Kajiwara, et al., 2008). Photodegradation is also observed for non-PBDEs flame retardant. Eriksson et al. (2004b) reported that TBBPA is susceptible to photolytic degradation in water. More limited data are available on the photodegradation of HBCD. A theoretical study of the photochemical properties of α-, β-, and γ-HBCD was done by Zhao et al. (2010) which predicted their photodegradation trends under the UV radiation, particularly at wavelengths shorter than 240 nm, however, which does not occur in the troposphere.

Biodegradation

It was reported that halogenated compounds could undergo metabolic processes, including oxidative or reductive dehalogenation, enzyme-catalyzed biotransformation, and other reactions (Fetzner, 1998). Thus, biodegradation could be one of the most significant processes for BFRs elimination in the environment. In general, the removal of the halogen

group is the key reaction during the biodegradation of halogenated compounds (Janssen et al., 2001), which can be based on different mechanisms (Smidt and de Vos, 2004). Reductive dehalogenation (i.e., replacement of halogens by a hydrogen or hydroxyl groups) is one important pathway, where halogenated compounds serve as electron acceptors in respiratory (halorespiration) processes and co-metabolic transformation, or in fermentative metabolism, in which a dehalogenated intermediate serves as electron acceptor (Fetzner, 1998; Janssen et al., 2001). Enzyme-catalyzed mechanisms, which are mediated by a wide range of microorganisms, can occur under both aerobic and anaerobic conditions. Aerobic biodegradation takes place predominantly in surface waters and soils. In contrast, anaerobic processes can occur in groundwater and sediments.

Biodegradation of BFRs has been reported in few studies in different environmental media under varying conditions. Biodegradation studies by Fackler (1989) for TBBPA in several media (water, sediment, and soil) showed that TBBPA was partially degraded under both aerobic and anaerobic conditions at different degradation rates (Han et al., 2008). In contrast, no degradation was observed for TBBPA tested in sewage sludge from Japan under sewage treatment conditions during 14 d incubation time (Han et al., 2008). Voordeckers et al. (2002) studied the anaerobic biodegradation of TBBPA in enriched sediments under different anaerobic environments. An almost completely removal of TBBPA was measured under both methanogenic and sulphate-reducing conditions with half-live (t½) of 55 and 112 d, respectively. A similar study was done by Arbeli et al. (2006) that aimed to examine the physiology of the microorganisms that reductively debrominate TBBPA. Davis et al. (2005) investigated biodegradation of HBCD in soil and sediment under both aerobic and anaerobic conditions. The study demonstrated that microorganisms naturally occurring in soil and sediment can facilitate a complete debromination of HBCD, with t½ of 63 and 6.9 d in aerobic and anaerobic soils, respectively. While in sediments, the t½ ranged from 11 to 32 d and 1.1 to 1.5 d under aerobic and anaerobic conditions, respectively. Biodegradation studies have also shown a potential breakdown of deca-BDE to lower brominated congeners, mainly nona-, octa-, and hepta-BDEs. In general, biodegradation appears to occur at much slower rates than photodegradation. In-vivo debromination of deca-BDE has been shown to occur (Mörck et al., 2003; van den Steen et al., 2007). Anaerobic degradation was also considered as a possible degradation process, particularly in soil, sediment, and sludge. He et al. (2006) studied degradation of deca-BDE by using pure bacterial cultures and cultivated them in a bicarbonate-buffered mineral salts media. Deca-BDE was almost completely degraded within two months, and octa- and hepta-BDE were detected. Biodegradation studies of deBDethane are still very limited. The degradation behaviour of deca-BDE may provide some indications for deBDethane considering their structural resemblances.

1.3.5 Bioaccumulation and toxicity

Concerns about the persistent character of compounds arise mainly from the combination with bioaccumulative and/or toxic properties. Criteria for a bioaccumulative compound according to POPs protocol (UNEP, 2001) are a bioconcentration factor (BCF) >5,000 in aquatic species, or high accumulation in other species, or in absence of measured data, log Pow >5. While the potential for adverse effects on human health or to the environment is a toxic compound criterium according to POPs protocol.

Bioaccumulation

PBDEs are expected as a bioaccumulative substance according to their high log Pow value (>5). However, the bulky structure of bromine groups of higher brominated BDEs probably produces an inhibition effect for their transport across biological membranes. Thus, deca-BDE has been regarded as not bioaccumulative (de Wit, 2002; ECHA, 2002; ECHA, 2006). In contrast, lower brominated PBDE congeners (i.e. four to seven bromines) are relatively more bioaccumulative with bioconcentration factors >5,000 (Birnbaum and Staskal, 2004).

A reliable BCF data for deBDethane is limited. A low level of accumulation was expected in fish by analogy with the analog substance deca-BDE. A worst-case interpretation of the in-vivo fish test data give an upper limit to the BCF of 1,600, assuming that the fish were exposed at the apparent water solubility limit, and that the concentrations in fish relate to internal tissues (rather than skin and gut contents). There is some uncertainty about the actual water solubility value of deBDethane, and if lower, the worst case BCF would be higher (Dungey and Akintoye, 2007).

Other non-PBDE BFRs demonstrated their bioaccumulative properties. The bioaccumulation potential of TBBPA in an aquatic environment directly links to the proportion of undissociated form, which predominates at lower pH values. TBBPA bioaccumulation data from several species of fish and aquatic invertebrates suggest a low to moderate potential for bioaccumulation. A study in fathead minnow, *Pimephales promelas*, reported BCF of 1,200 (Environment Canada, 2013). Uptake and accumulation of TBBPA in the earthworm, *Eisenia fetida*, was also investigated in the concentration range of 0.5 to 35.4 mg/kg soil d.s. A BCF of 5.1 was obtained for earthworms at the lowest test concentration while for the remaining of concentrations it ranged from 0.24 to 0.019 (Environment Canada, 2013). HBCD bioaccumulation studies by Drottar et al. (2001) in rainbow trout, *Oncorhynchus mykiss*, reported BCF >5,000. There are no earthworm BCF studies available. However, BCF ranged

from 0.03 to 0.08 (based on soil and earthworm wet weight) was shown in the study on *Eisenia fetida* exposed to HBCD (Aufderheide et al., 2003).

Morris et al., (2004) reported field bioaccumulation data for TBBPA and HBCD. Both compounds were measured in species representing different trophic levels from the North Sea aquatic food webs: harbor porpoises (*Phocoena phocoena*), seals (*Phoca vitulina*), cormorant (*Phalacrocorax carbo*), and eels (*Anguilla anguilla*). There is evidence that biomagnification of HBCDs is occurring through these webs. However, TBBPA shows less bioaccumulative potential than HBCD. The more polar and reactive molecular properties of TBBPA might result in this lower degree of bioaccumulation.

Toxicity

BFRs' properties as endocrine-disrupting compounds (EDCs) cause concerns as well. EDCs have an ability to mimic or block the action of thyroid and other endocrine hormones, due to their structural similarities. TBBPA has been demonstrated as a thyroid hormone agonist but its disrupting effect on estrogen signaling is relatively weak (Meerts et al., 2001). TBBPA can also act as a cytotoxicant, neurotoxicant, and immunotoxicant (Canesi et al., 2005). TBBPA is readily absorbed from the gastrointestinal tract, but rapidly metabolized and excreted via bile acid (Schauer et al., 2006). The acute toxicity of TBBPA is reported as very low, and there is no evidence for reproductive or teratogenic effects in human (EFSA, 2011). Like TBBPA, HBCD has adverse effects on the thyroid hormone system and induce genetic alterations in mammalian cells (Helleday et al., 1999). HBCD was also associated with changes in spontaneous behavior, learning, and memory defects (Birnbaum and Staskal, 2004; Eriksson et al., 2006).

In general, PBDEs toxicity depends on the bromine content of the corresponding congeners. Their toxic potential decreases in the order of penta-BDE > octa-BDE > deca-BDE (Darnerud et al., 2001). In contrast to penta- and octa-BDE, the bulky molecule of deca-BDE is poorly absorbed, and thus less toxic. Acute toxicity of all PBDE congeners were reported to be low (Rahman et al., 2001; Hardy, 2002). The substantial concerns in relation to PBDEs toxicity are particularly neurotoxicity effects, which were identified in animal studies (Birnbaum and Staskal, 2004). PBDEs are also considered as endocrine disruptor, particularly for penta- and octa-BDE congeners (Meerts et al., 2001; Vonderheide et al., 2008). PBDEs are suspected as carcinogens. However, there is no clear evidence currently available (Siddiqi et al., 2003; Vonderheide et al., 2008).

Information on the toxic effects of deBDethane is still very limited. In general, deBDethane is reported having a relatively low toxicity and no effects were observed during acute oral and dermal tests on rat and rabbit, respectively (Dungey and Akintoye, 2007). Chronic tests for sediment-dwelling organisms did not produce any observable effects (Hardy et al., 2012). Similar results were found on acute and chronic tests on some aquatic species (fish, algae, and daphnia) (Hardy et al., 2012).

1.3.6 Regulation

The use of PBDEs in electronic equipment has been restricted in the EU since 2006 under the RoHS (Restriction of Hazardous Substances) Directive. On 1st April 2008, the European Court of Justice annulled the deca-BDE exemption to the RoHS Directive granted by European Commission. Deca-BDE was registered under REACH (Registration, Evaluation, Authorization and Restriction of Chemical) at 2010. In USA and Canada, deca-BDE will be voluntarily withdrawn in all applications by producers (Chemtura and Albemarle) by the end of 2013 (USEPA, 2010b). However, production and use of deca-BDE, which is currently represent 30% of global BFRs consumption (BSEF, 2003), seems to continue in other regions (Birnbaum and Bergman, 2010).

HBCD is one alternative for additive PBDEs in many applications (Covaci, et al., 2006). However, several initiatives have been proposed to assess and regulate the use of HBCD (Kemmlein, et al., 2009; Kjeldby, 2011). HBCD is recently under regulation in the US (USEPA, 2010a) and Canada (BSEF, 2012b). In 2007, the European assessment, which is based on 793/93/EC Directive on the evaluation and control of the risks of existing substances, concluded that HBCD met PBT criteria (ECHA, 2008a; BSEF, 2012b). Subsequently, in 2010 HBCD was registered under REACH and classified as substances of very high concern (SVHC) (BSEF, 2012b). HBCD is also covered by the emissions control programs (VECAP and SECURE) as an efforts by the HBCD industry to control its release into the environment (Kemmlein, et al., 2009; BSEF, 2013b). More recently, in 2010 HBCD has been proposed to be listed as POP (SCOP, 2012), however with specific exemptions for its production and application in EPS and XPS in buildings.

In contrast, so far there are no legal barriers for the production and application of TBBPA or its derivatives and deBDethane. The use of TBBPA is not subject to any regulatory restriction in USA, EU, or in other countries (BSEF, 2012a). As HPVC, TBBPA was listed in 2003 in the 2002/96/EC Directive on the collection and recycling of Waste of Electrical and Electronic Equipment (WEEE), which implied a selective treatment for TBBPA-containing products, and

was subsequently registered under REACH in 2010. TBBPA is not included in the substances restricted by the RoHS Directive even following the latest revision in 2010 (BSEF, 2012a). A current legislative control does not exist for deBDethane. So far, a few reviews have been initiated by regulatory authorities. In Europe, releases of deBDethane are restricted under WEEE Directive (Dungey and Akintoye, 2007). This compound has been also covered by emission control program VECAP. In Canada and USA, deBDethane is under regulation to control its release to the environment, i.e. Canadian Environmental Protection Act (CEPA) and US Toxic Substances Control Act (TSCA), respectively. A review by German Federal Environmental Agency recommended further studies that should be done before a final conclusion on the risk of deBDethane can be drawn (UBA, 2001).

1.4 Treatment of sewage sludge

Sludge treatment, which is included in the process of WWTPs, must be performed prior to disposal and usage of sewage sludge (i.e. agricultural purpose) in order to meet the quality guidelines. The main aims of sludge treatment are to reduce sludge volume and to stabilize its organic material, hence to prevent further decomposition and to meet hygienic standards. The most common treatment options for sludge treatment include stabilization by drying, anaerobic and aerobic digestion.

1.4.1 Stabilization by drying

Drying of sewage sludge is a reliable process for sewage sludge treatment because it results in significant mass and volume reduction and, consequently, in cost savings concerning sludge storage, handling, and transport (Chen et al., 2002; Bennamoun, 2012). However, drying is a very energy intensive process, hence utilizing free solar energy become a preferable option. Sludge stabilization by solar drying is widely applied in arid countries characterized by a high annual solar radiation. Application of this method in several countries have been reported, such as USA (El-Ariny and Miller, 1984), Egypt (Hossam et al., 1990), Australia (Gharaibeh et al., 2004), Jordan (Radaidah and Al-Zboon, 2011), and Greece (Mathioudakis et al., 2013). This technology is especially a choice for developing countries as it is lower in operational costs and less sophisticated in operation and maintenance with a minimum requirement of expertise (Bennamoun, 2012).

Raw sludge is characterized by very high water content ranging from 90 to 99% and distributed in different forms (**Fig. 1.4**) (Chen et al., 2002): (1) free water, that is not attached to the sludge particles and can be removed by gravitational settling; (2) interstitial water, that

is trapped within the flocs of solids or exists in the capillaries of the dewatered sludge and can be removed by strong mechanical forces; (3) surface water, that is held on the surface of the solid particles by adsorption and adhesion; and (4) intracellular and chemically bound water. Under constant drying process, free water will be removed firstly, followed by the interstitial water, and finished by surface water. The final water content retained within the sludge is mostly chemically bound water in amounts that depend on the type of sludge and the drying conditions (Chen et al., 2002).

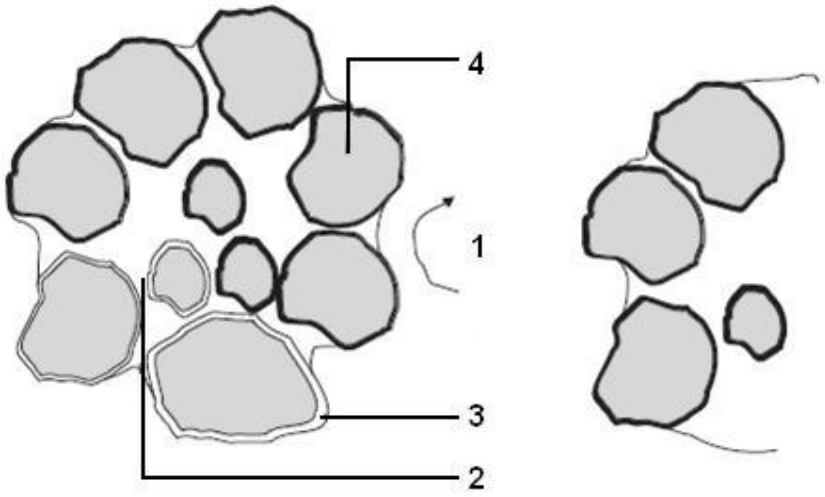


Figure 1.4: Water distribution in sludge: (1) free water, (2) interstitial water, (3) surface water, (4) intracellular and chemically bound water (Chen et al., 2002)

Sludge drying is performed in solar plants or under open air. Solar plants are designed as greenhouses that are constructed from a transparent material, and a floor, where the sludge is speared in thick layers (Bennamoun, 2012). Sludge drying in such solar plants accelerates the water evaporation rate by exploiting the artificial greenhouse effect and avoiding the equilibrium of vapor pressure between sludge and air by controlled indoor air ventilation. Thus, the energy requirements are less than in the case of open air dryers (Bux et al., 2002; Chai, 2007; Vijayavenkataraman et al., 2012). In open drying beds, periodical turning the sludge increases the drying rate, decreases the insect populations, and reduces the odor problem through increased aeration and the disturbed incubation of insects. Furthermore, it prevents the formation of thin surface layers, which would inhibit the drying of the remaining sludge (Chen et al., 2002).

The drying by sun depends on the weather conditions. Mathioudakis et al. (2013) reported for a pilot-scale sludge solar drying in Greece (40°N) that a period of 9-18 d was sufficient in

order to produce sludge with up to 95% d.s. during summer conditions (May to September). An improvement of sludge quality was also reported in this study. The solar drying process resulted in partial sludge sanitation as total and fecal coliform contents were reduced up to three orders of magnitude, and the sludge organic content, (expressed as volatile solids), was also slightly decreased. This partial sludge disinfection may be attributed to the exposure of microbes to solar ultraviolet radiation and elevated sludge temperatures, with simultaneously reduced sludge moisture content. By the end of the drying period, the sludge fecal coliform content was found to be below 2×10^6 CFU/g d.s., which met the limit value of the EPA Class B pathogen requirement. This value indicates that pathogens were reduced to levels that protect public health and the environment.

1.4.2 Anaerobic digestion

During anaerobic digestion, organic sludge matter is decomposed microbiologically in the absence of oxygen and converted to methane (CH_4), carbon dioxide (CO_2), and other inorganic end products. Anaerobic digestion allows an effective treatment of a variety of sludges from different wastewater categories, such as industrial effluent (Macarie, 2000), domestic sewage (Elmitwalli et al., 2001), vegetables waste (Bouallagui et al., 2005), and also applicable for the stabilization of solid waste (Sharma et al., 2013). An average of 70% of the solids present in typical domestic wastewaters are organic origin, out of which approximately 40 to 60% are proteins, 25 to 50% are carbohydrates, and 10% are fats and oils. Some amounts of urea, surfactants, phenols, pesticides, and other chemicals can also be found (Borges and Chernicharo, 2009). A wide range of microorganisms, mainly methanogens, are involved in anaerobic digestion and the conversions of complex organic materials into simple matter is accomplished in four basic stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (**Fig. 1.5**).

Hydrolysis

The anaerobic digestion process is initiated by the hydrolysis step. This step involves an enzyme-mediated conversion of complex organic polymers (polysaccharides, lipids, protein, nucleic acids) to simple monomers (monosaccharides, fatty acids, amino acids) which are further used by bacteria for fermentation. During this process, complex insoluble or soluble organic molecules are first reduced in size to facilitate the transport of molecules through the cell membranes. Exo-enzymes released by facultative and/or obligate fermentative bacteria to the medium are responsible for the solubilization and size reduction process of those organic molecules. Carbohydrates are hydrolysed by amylase to mono- and disaccharides,

lipids by lipase to glycerol and fatty acids (Mackie et al., 1991), and proteins by protease and peptidase to amino acids (Ramsay and Pullammanappallil, 2001).

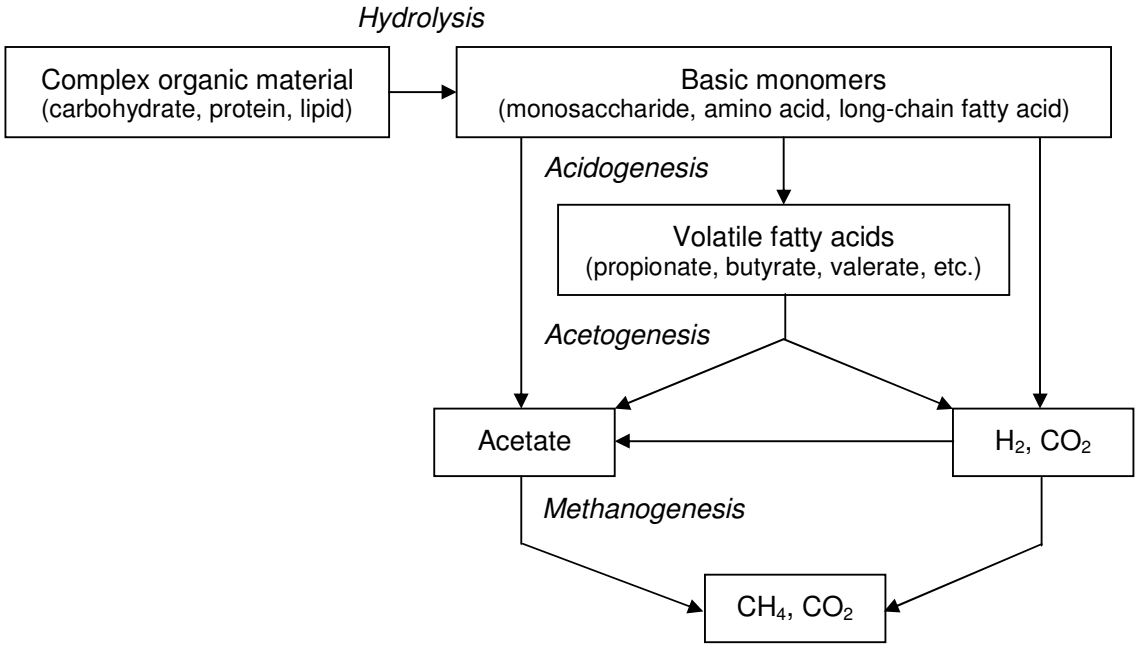


Figure 1.5: Schematic diagram of metabolic steps in anaerobic digestion (redrawn from Ersahin et al., 2011)

Acidogenesis

The next step after hydrolysis is the acidogenesis. During this step, small molecules (monosaccharides, fatty acids, and amino acids) produced by hydrolysis are converted to volatile fatty acids (VFA), alcohols, ketones, etc., along with CO_2 and H_2 by acidogenic bacteria. Acidogens represent the largest group of anaerobic bacteria, comprising 90% of the total population (Zeikus, 1980). The following reaction (Eq. 1.2) shows the acidogenesis reaction where glucose serves as substrate. Products are respectively H_2, CO_2, ethanol, and acetic, propionic, and butyric acid (Fernández et al., 2011).

$$C_6H_{12}O_6 \rightarrow H_2 + CO_2 + C_2H_5OH + CH_3COOH + CH_3CH_2COOH + CH_3CH_2CH_2COOH \quad (1.2)$$

Acetogenesis

VFA produced during acidogenesis that are larger than acetic acid, like propionic and butyric acid, are further converted to acetate, CO_2, and H_2 (Angelidaki et al., 2011). The process is known as acetogenesis by acetate and H_2-producing bacteria that are collectively termed acetogenic bacteria. These groups of bacteria require low H_2 partial pressure from fatty acid

conversions. Thus, under high H_2 tensions acetate formation is reduced. Methanogens help to maintain a low level of H_2 partial pressure by extensive utilization of H_2 to produce CH_4. Therefore, the H_2-producing bacteria and methanogens are known to have symbiotic relationships (Grady et al., 1999).

Methanogenesis

The products formed by non-methanogenic processes (acidogenesis and acetogenesis) (i.e. formic acid, acetic acid, CO_2, and H_2), are further used by a group of microorganisms which are collectively known as methanogens in order to produce CH_4. Methanogens are member of the domain *Archaea*, which synthesize CH_4 as the major product of their energy metabolism (Whitman et al., 2006). Methanogenic organisms can be distinguished into two groups: acetoclastic methanogens, which convert acetate into CH_4 and CO_2 (Eq. 1.3) and H_2-utilizing methanogens, which use hydrogen as the electron donor and CO_2 as the electron acceptor to produce CH_4 (Eq. 1.4) (Demirel and Scherer, 2008). Acetoclastic reaction represents the common pathways of the CH_4 production, around two-third of total methanogenesis (Gujer and Zehnder, 1983).

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (1.3)$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (1.4)$$

During anaerobic digestion, the four processes (hydrolysis, acidogenesis, acetogenesis, and methanogenesis) occur simultaneously. However, this system suffers from problems of process stability, particularly concerning methanogenesis. In a properly working anaerobic digester, the production of VFA by fermentation reaction and their utilization rates by methanogen are in equilibrium. If process disturbances occur and the methanogenic organisms do not fast enough to use the produced H2, the fermentation of propionate and butyrate will be slowed by the accumulation of VFA in the anaerobic reactor. In consequence, FVA will accumulate. Furthermore, acid levels will continuously rise due to the robust nature of the acid forming bacteria, pH will quickly decrease, and CH4 production will be inhibited at pH values 5.5 to 6.25 (Staley et al., 2011). Finally, this leads to a stop of the anaerobic process.

Anaerobic digestion can be run at mesophilic (30-40 °C) or thermophilic conditions (50-60 °C) (van Lier et al., 1996). The mesophilic process has been more in use for sludge stabilization because it requires less energy input for heating (Gallert and Winter, 1997). Furthermore, it has higher process stability because it is less affected by inhibitory

compounds, such as ammonia (Sánchez et al., 2000). Accumulation of propionate during the thermophilic process were, to some extent, also greater than in the mesophilic process (Nosrati et al., 2011). On the other hand, increased temperature under thermophilic condition generally favours the metabolic activity of the microorganism (Zábranská et al., 2000; Kardos et al., 2010) and a higher sanitation effect is achieved (Zábranská et al., 2000; Sahlström, 2003). Reduced viscosity of the sludge and increased diffusion rates of the substrate that usually lead to higher reaction rates are further advantages of the thermophilic process (Holst et al., 1997).

The main benefits of the anaerobic sludge treatment compared to the aerobic process are the production of CH_4, which can be used to heat the reactor and maintain its performance. This process also produces smaller amounts of biosolids at the end of the process, because the biomass growth is much lower compared to those in aerobic processes. Anaerobic digested sludges are much more compact than aerobic biosolids. Furthermore, anaerobic digestion process effectively reduces and inactivates pathogens (Dahab et al., 1996; Epstein, 2003; Côté et al., 2006).

1.4.3 Aerobic digestion

Aerobic digestion is a process under aerobic conditions involving aerobic microorganisms which is applied to degrade or convert organic matters to carbon dioxide, water, and biomass (microorganisms). The aerobic digestion process consists of two steps (Zupančič and Roš, 2008): (1) direct oxidation of biodegradable matter, and (2) endogenous respiration in which cellular material is oxidized, as described below.

During the oxidation process, aerobic and facultative microorganisms use oxygen to gain energy from the available biodegradable organic matters in sludge. These organic matters are broken down to CO_2 and H_2O in order to gain energy for the production of microbial biomass (cellular material) (Eq. 1.5). However, when the available nutrients in sludge are inadequate, the microorganisms start to consume their cell contents for metabolic purposes. Thus, under substrate-limited conditions the cells undergo lysis, and the cellular material is oxidized aerobically to CO_2, H_2O, and degradable organic matter (Eq. 1.6). The process is called endogenous respiration and is the predominant reaction in aerobic digestion systems. If such conditions are maintained over an extended period, the total amount of biomass will be significantly reduced. Furthermore, the remaining material will exist in a low energy state and can be considered biologically stable and suitable for further disposal to the environment.

$$\text{organic matter} + NH_4^+ + O_2 \rightarrow \text{cellular material} + CO_2 + H_2O \quad (1.5)$$

$$\text{cellular material} + O_2 \xrightarrow[\text{aerobic bacteria}]{} \text{digested sludge} + CO_2 + H_2O + NO_3 \quad (1.6)$$

In the aerobic system, approximately 75 to 80% of cellular material can be oxidized and the remaining mass is called nondegradable materials, which mainly consists of cell walls and other cell fragments (Metcalf and Eddy, 1991). Primary sludge typically contains little cellular material. Thus, addition of primary sludge in the process can shift the overall reaction to Eq. 1.5, resulting in an increase of total biomass. Consequently, the aerobic digestion process, in which the sludge mass is reduced, is only recommended for excess activated sludge. For primary sludge digestion, anaerobic digestion is preferred (Zupančič and Roš, 2008).

The most critical parameter in the aerobic digestion process is dissolved oxygen (DO) parameter. Adequate oxygen concentrations allow the biological process to occur while inadequate DO levels result in an incomplete digestion process and lead to a formation of odors. After aeration starts, the aerobic process can be divided into four phases (Buchanan and Seabloom, 2004) (**Fig. 1.6**): (1) lag phase, microorganisms are adapting themselves to the aeration environment (little biomass production); (2) growth phase with rapid growth of aerobic bacteria (high demand of O_2); (3) stationary phase, the end of growth period (moderate oxygen demand and the onset of cell autolysis); and (4) endogenous phase, cell destruction increases (low demand of O_2).

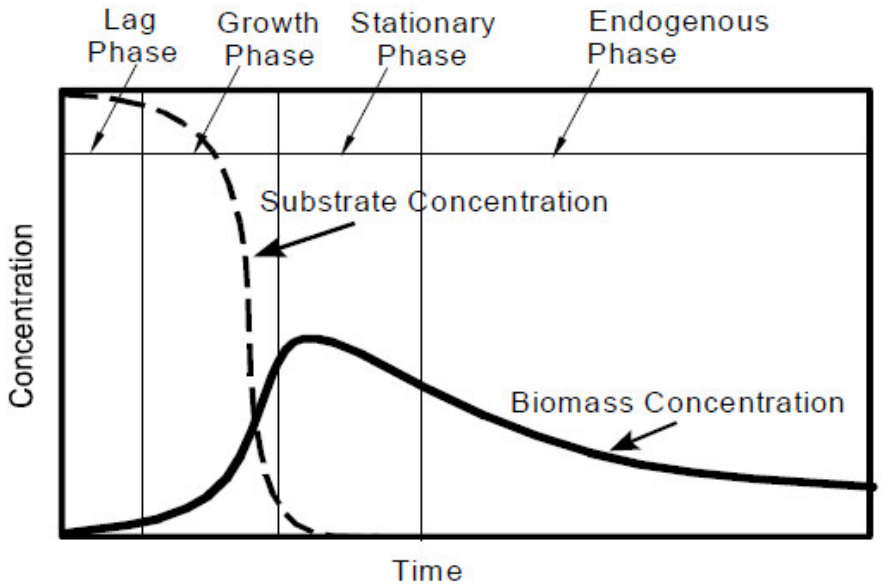


Figure 1.6: Kinetics of aerobic digestion showed a biomass concentration changes in response to changes in substrate concentration (Buchanan and Seabloom, 2004)

Aerobic digestion can occur in a temperature range from ambient to thermophilic. Some authors report of the optimal temperature for nitrification, a key step in the digestion process, as 30-35 °C (Neufeld et al., 1986; Antoniou et al., 1990; Willers et al., 1998). In most aerobic digesters, the temperature is a function of ambient weather conditions and is not controlled. Thus, temperature variability throughout the year may cause variability in the operating performance of the aerobic process.

Aerobic digestion has some advantages in comparison to anaerobic digestion, which are: easy start up, excellent ammonia and BOD removals, materials with less odor, and simplicity of the system operation and maintenance. While disadvantages of the system are: no useful byproduct, such as CH_4 gas, less reduction in organic matter as the result of high biomass production, more difficult in dewatering of stabilized sludge, and higher energy cost for aeration.

1.5 Chemical analysis of BFRs

Analytical methods for BFRs in environmental matrices were described by many authors, e.g. de Boer et al., 2001; Hyoëtylaëinen and Hartonen, 2002; Eljarrat and Barceló, 2004; Kierkegaard et al., 2004; Covaci, et al., 2007; Díaz-Cruz et al., 2009; Dirtu, 2009. However, most studies are focused on aqueous matrices (e.g., surface water and sewage water) and few methods have been developed for solid matrices, as sewage sludge. In general, the analytical techniques for BFRs analysis has a similar approach to other organohalogen compounds which has been extensively reported, such as OCs and PCBs (e.g. Kolb et al., 1995; Andreu and Picó, 2004; Tadeo et al., 2010) and PBDD/Fs (e.g. Ebert et al., 1999; Ebert and Bahadir, 2001).

Sewage sludge is a challenging matrix because of the complexity of its composition (Díaz-Cruz et al., 2009). Sewage sludge contains diverse of other components that are potential interferences in analyzing the compounds of interest. Those co-extracted interferences include lipids, organic acids, humic substances, and other naturally-occurring materials, as well as lipophilic materials that may be added to the sewage during processing (e.g., hydrocarbons, dyes, surfactants, polymeric colloids, etc.). These components possible to manifest themselves as interferences at all steps of the analytical procedures. This aspect also has to be considered in developing sufficient clean-up procedures (Díaz-Cruz et al., 2009).

1.5.1 Sample pre-treatment

Sample pretreatment is a crucial step in the analytical process. Solid matrices (soil, sediment, sewage sludge) have to be water-free and should be dried before extraction to enable extraction of the analytes when non-polar solvents are used. Lyophilization or freeze-drying has been widely used (de la Cal et al., 2003; Yusa et al., 2006; Sanchez-Brunete et al., 2009; Wang et al., 2009) for water removal from the matrices. Mixing the sample with anhydrous Na_2SO_4 (Morris et al., 2004; Labadie et al., 2010) is another option. Subsequently, thorough sieving, grounding, and mixing have to be done in order to ensure sample homogeneity.

1.5.2 Extraction

A variety of extraction methods has been used for the extraction of BFRs from environmental samples. Soxhlet is one of the most frequently used techniques (Morris et al., 2004; North, 2004; Remberger et al., 2004; Knoth et al., 2007; Eljarrat et al., 2008) and has been adopted in many standardized analytical methods for its general robustness and relatively low costs. However, advanced extraction techniques have recently been introduced, such as pressurised liquid extraction (PLE) (La Guardia et al., 2007; Ricklund et al., 2008a; 2008b), solid-phase microextraction (SPME) (Montes et al., 2010), matrix solid-phase dispersion (MSPD) (Sanchez-Brunete et al., 2009), ultrasonic-assisted extraction (UAE) (Labadie et al., 2010), and microwave-assisted extraction (MAE) (Shin et al., 2007). The main advantage of those methods is the reduced extraction time and solvent consumption.

Both single and binary solvent systems are in use for the extraction of BFRs from solid matrices. For Soxhlet extraction, mixture of solvents in different proportions, such as n-hexane/acetone (1:1 v/v) (Morris et al., 2004; Chen et al., 2009), n-hexane/acetone (3:1 v/v) (Allchin et al., 1999; de Boer et al., 2003; Morris et al., 2004; Kupper et al., 2008), n-hexane/dichloromethane (DCM) (1:1) (Eljarrat et al., 2008) have been widely used. Application of single solvent has also been reported, such as n-hexane (Hyoëtylaëinen and Hartonen, 2002), toluene (Hagenmaier et al., 1992; Knoth et al., 2007), acetone (Remberger et al., 2004), DCM (North, 2004). The Soxhlet extraction time is varied from 4 to 72 h (Allchin, et al., 1999; Hagenmaier et al., 1992; de Boer et al., 2003; Morris et al., 2004; Chen et al., 2009) which depends on test compound, matrix, and solvent composition.

1.5.3 Cleanup

The non-selective nature of the extraction procedures and the complexity of the sludge matrices result in complex extracts that require further purification steps prior to BFRs analysis. Therefore, cleanup procedures have to be applied in order to remove those interfering co-extractants that are present in the raw extracts.

Cleanup techniques for sludge matrices, which often give high amounts of co-extractants, can be based on destructive and non-destructive methods. Treatment with concentrated sulphuric acid as a destructive cleanup method was widely applied (Hagenmaier et al., 1992; Nylund et al., 1992; Öberg et al., 2002; de Boer et al., 2003; Morris et al., 2004; Eljarrat et al., 2008). Non-destructive cleanup methods, such as gel permeation chromatography (GPC), are preferred for compounds that are not stable in the presence of strong acids. Application of GPC for the removal of high molecular organic co-extractants by separation based on molecular size was reported in several publications, e.g. Hagenmaier, et al. 1992; de Boer et al., 2003; Morris et al., 2004; Knoth et al., 2007; Kupper et al., 2008.

Column chromatography with a variety of absorbents (i.e., silica, florisil, and alumina) is also a widely applied as a standard technique for BFRs clean-up. Activated and deactivated absorbents (i.e. silica) are used. The former is prepared by heating the absorbent at 160 °C for several hours while deactivated silica typically contains up to 20% water (Patnaik, 1997). Column cleanup techniques are also based on single or multiple layer columns containing varying amounts of neutral, acid, and basic adsorbents, with different degrees of acid or base impregnation. Both destructive and non-destructive impregnations were successfully applied in column chromatography. Impregnating silica gel with strong acids as H_2SO_4 was frequently used in the analysis of BFRs (de Boer et al., 2001; Kierkegaard et al., 2004; Covaci et al., 2005). Multi-layer columns with different combinations of neutral, acidified, and basic silica were also used (Amakura et al., 2002; Huwe et al., 2002; Mai et al., 2005; Vorkamp et al., 2005; Konstantinov et al., 2006; Ebert and Bahadir, 2007). Unpolar organic solvents, such as n-hexane and a little more polar mixtures of n-hexane/DCM, were widely used as eluting solvent. In order to ensure a water-free extract, anhydrous Na_2SO_4 is often added to the top of the multi-layer column before elution with the solvents (Covaci et al., 2011b).

Sewage sludge extracts often contain relatively large amounts of elemental sulphur because of the anaerobic conditions of the matrix. Sulphur in the matrix interferes the analysis of BFRs by GC-MS. Treatments with tetrabutylammonium sulfite reagent (Kolb et al., 1995), Cu

powder (de Boer, et al., 2001) or by GPC (Morris et al., 2006) are efficient approaches for sulphur elimination.

1.5.4 Identification and quantification

HPLC/DAD and LC/MS/MS

High performance liquid chromatography (HPLC) is a common method for BFR analysis (Riess and van Eldik, 1998; Schlummer et al., 2005; Köppen et al., 2006; Haug et al., 2007). HPLC is applicable for a wide range of polarity and acidity and is suitable for thermolabile compounds, as there are no thermally induced reactions during HPLC elution. Higher brominated PBDEs, such as BDE-209, are characterised by high boiling points and require very high injection and elution temperatures (300 °C), which lead to formation of artefacts due to thermal degradation (Schlummer et al., 2005). Furthermore, HPLC allows the isomer-specific determination of HBCD (Budakowski and Tomy, 2003; Suzuki and Hasegawa, 2006; García-Valcárcel and Tadeo, 2009), while by GC-MS analysis only total HBCD can be measured (Law et al., 2005; Abdallah et al., 2008). As TBBPA is a phenolic compound and thus relatively polar, its determination by HPLC is the simplest and the best option (de Boer and Wells, 2006). TBBPA analysis by GC-MS needs derivatization, i.e. with acetic anhydride to the acetylated derivative, in order to obtain an adequate response and avoid peak tailing (Sellström and Jansson, 1995).

Both reversed phase (RP) and normal phase (NP) separation have already been applied in the analysis of BFRs (Cariou, et al., 2006). The NP system is more compatible for the PBDEs because of their solubility properties. They can only be solved in very unpolar solvents as hexane, THF, etc. For detection, HPLC coupled with ultraviolet (UV) detection (HPLC/DAD) have been widely applied (Riess and Eldik, 1998; Schlummer et al., 2005; Pöhlein et al., 2005). In this method, the identification of test compound present in the samples is mainly based on a comparison of the peak retention times (t_R) and the corresponding UV absorption spectra with those of known standards. A characteristic sequence of peaks (peak pattern recognition) is also useful for the identification of certain technical BFRs (Riess and Eldik, 1998). However, HPLC/DAD is preferred to polar rather than non-polar samples and shows a limited detection limits and specificity as compared to MS detection with the analytical range of ng/µL. Recent developments using LC/MS/MS based on different ionization modes (Electrospray ionization = ESI, Atmospheric pressure chemical ionization = APCI, and atmospheric pressure photoionization = APPI) are reported and compared with those obtained by GC-MS techniques (Saint-Louis and Pelletier, 2004; Petersen et. al., 2004; Bacaloni et al., 2009).

GC/MS

GC has become a routine technique for the analysis of PBDEs. Due to their physicochemical properties (e.g. vapour pressure or polarity) and to their thermal instability, specific PBDE congeners need special precautions during GC analysis. It was shown that thermal degradation might occur during GC analysis of higher brominated PBDE congeners (Roosens et al., 2008; Dirtu et al., 2009; Kierkegaard et al., 2009). Thus, for PBDEs analysis, characteristics of the GC system are very critical aspect and should carefully be optimized concerning the properties of the compounds. These characteristics include column dimensions (i.e. column length, internal diameter), stationary phase, injection technique, and temperature program. Those parameters have a significant influence on the accuracy of PBDE analysis (Björklund et al., 2004; Covaci et al., 2007). Finally, the detection technique is another important parameter that has to be optimized for an accurate determination of PBDEs.

The choice of a GC column for PBDEs analysis requires consideration of both the separation power of the column and thermal degradation of the analyte on the column. Capillary GC columns offer an adequate resolution of individual PBDE congeners. A sufficient length (30–50 m) and small internal diameters (≤0.25 mm) non-polar or semi-polar column are preferred to achieve adequate separation of lower PBDE congeners (Kierkegaard et al., 2009). However, higher brominated congeners (e.g. BDE-209), which need high evaporation temperature and thus can be thermally degraded at that temperature, requires other instrumental conditions. In general, in order to reduce the thermal degradation of analytes, the residence time in the column as well as in the injector should be minimized (Björklund et al., 2004). It was also shown that a low elution temperature (<295 °C), combined with a short residence time in GC lead to minimal thermal degradation (Dirtu et al., 2008). Thus, a short column (≤15 m) with a thin stationary phase (0.1 µm) is a good option to reduce the interaction time with the stationary phase (Kierkegaard et al., 2009).

The injectors usually applied for PBDEs analysis are hot vaporizing injectors such as splitless, pulsed splitless, programmable temperature vaporization (PTV), or cold on-column. Due to the relatively low levels of PBDEs in environmental samples, splitless injection is the preferred technique, but special precautions have to be taken to minimize thermal degradation and discrimination of higher molecular weight PBDEs. Therefore, the optimal injector temperature and splitless time should be kept as high as possible to obtain an increased response factor especially for fully brominated PBDE (Björklund et al., 2004). An alternative to reduce thermal degradation of PBDEs in injection system is to apply on-column injection. This technique delivers the extract directly to the column, thus shortcuts the injector

interface between extract and column. As a result, higher precision for the analysis of analytes was observed (Björklund et al., 2004). However, this technique need a clean sample extracts to avoid potential contamination of the column by matrix residues, which is possible to produce increased noise, peak tailing, retention time shifts, column trimming, and reduced column lifetime.

The most widely used detectors for PBDE determination are mass spectrometers, classified into low-resolution (LR) or high-resolution (HR) mass spectrometric (MS) instruments. The LR-MS instruments are operated either in electron impact (EI) or in electron capture negative ion (ECNI) mode (de Boer et al., 2001; Covaci et al., 2003; Korytar et al., 2005). GC-ECNI-MS is a very sensitive technique (Haug et al., 2008). However, GC-EI-LRMS provides a higher selectivity and accuracy in quantification (Eljarrat and Barceló, 2004). Its sensitivity for higher brominated PBDE congeners (>6 bromines) is particularly poor compared to GC-ECNI-MS. However, for lower brominated congeners (mono- and di-BDEs), GC-EI-LRMS shows better sensitivity (Ackerman et al. 2005). In EI-MS mode, the major ions formed are $[M^{\bullet}]^{+}$ and $[M\text{-}2Br]^{\bullet+}$ (Sellström, 1999), which can be used for for substance identification (using the scan mode), or for quantification (using the single ion monitoring (SIM) mode). The main potential interferences that may influence the accuracy come from chlorinated compounds, such as PCBs, which abundant in sludge matrices. For example, the nominal masses corresponding to ions monitored for di-BDEs and penta-CBs (m/z = 326), and also for tetra-BDEs and hepta-CBs (m/z = 396). Thus, a high resolution power is needed to separate them if they co-elute on the GC column (Covaci et al., 2007).

In the analysis of HBCD, GC has limitations because it only provides information about the sum of α, β, γ-HBCD diastereoisomers due to coelution and possible thermal interconversion of these diastereoisomers at temperatures >160 °C (Morris et al., 2004; Tomy et al., 2005; Covaci et al., 2007). Therefore, it was impossible of separating them. Moreover, HBCD isomers are thermally labile and decomposition can take place in the inlet and the column of GC-MS at temperature >240 °C. HBCD degradation happens by sequential elimination of HBr and results in a broad and splitted peak in the GC chromatogram, as also reported by Barontini et al. (2001) and Morris et al. (2006). This degradation can be reduced by lowering the temperature of the inlet (e.g. to 220 °C), as proposed by Frederiksen et al., (2009), thus reducing the intensity of degradation.

2. Motivation and Objectives

In the past few decades, the global production and application of BFRs for various industrial and domestic polymer-based products in order to improve their fire resistance has dramatically increased. Currently, more than 400,000 t of BFRs are used globally, and the use is still constantly growing. In parallel with their extensive usage, the elevated occurrence of BFR residues in various environmental compartments has also been measured. Thus, due to the widespread presence in the environment and their reported possible adverse health effects, BFRs has been a subject of environmental concern. Sludges provide a sink for hydrophobic compounds like BFRs, which tend to adsorb onto solids. Subsequent sludge disposal and application, for example as fertilizer in agriculture soil, became an important route for the distribution of BFRs into the environment. The fate of these persistent organic compounds following sewage sludge treatment is a topic of current concerns. Unlike other organic pollutants, which are completely or partially degraded in WWTPs, there are indications that BFRs are relatively unaffected by the treatment step in WWTPs, and thus persist in the sewage sludge. Inadequate elimination during sludge treatments, particularly the digestion step, is perhaps the main reason for a continuous release of those compounds to the environment. So far, relatively little is known about the persistence of BFRs during different sludge treatment processes.

Therefore, the aim of the study was to explore the fate and behaviour of selected BFRs during sludge treatments. For this purpose, BFRs with a high market share should be selected. A further criterion should be to choose substances with different molecular structures and chemical properties, in order to compare their structure behaviour relation. Three different treatments should be studied: aerobic digestion, anaerobic digestion, and the stabilization by drying under the sun. The dissipation rate of the test compounds should be evaluated at these three processes. In a further approach, a sequential anaerobic-aerobic treatment should be investigated by performing an anaerobic phase and followed by an aerobic phase in order to study the influence of the combination of both processes on the dissipation.

In order to realize these studies, fortification experiment should be performed in the lab-scale by using small batch test systems. Before starting the batch tests, analytical methods should be developed in order to quantify the dissipation of the test compounds during treatment. Furthermore, the sludge matrices in charge to be used for the experiments should be characterized by their background levels of the investigated BFRs. From the results of the batch tests, dissipation kinetics should be calculated. For the evaluation of the performance

of the batch test systems, various parameters should be measured and continuously monitored, including incubation temperature, pH, redox potential (E_h), C, N, and relevant nutrients (phosphate, nitrate) removal. For the anaerobic batch system, biogas production should be monitored. Finally, the treated sludge should be investigated for the formation of degradation products. For this purpose, GC/MS analysis had to be performed.

3. Materials and Methods

3.1 Test compounds

The test compounds were purchased from following providers: TBBPA from Riedel-de Haën (Seelze, Germany); HBCD and BDE-209 from Sigma-Aldrich (Milwaukee, USA); and the technical deBDethane, FireMaster® 2100, from AccuStandard (New Haven, USA). The purity of the test compounds tested by using HPLC/DAD method was ≥95%. Individual stock solutions were prepared in methanol (Fisher Scientific, Leicestershire, UK) for TBBPA and in THF (Merck, Darmstadt, Germany) for HBCD, BDE-209, and deBDethane. Each stock solution contained 1 µg/µL of the standard compound, except deBDethane for which a stock solution of 0.05 µg/µL was prepared due to its low solubility. These stock solutions were stored at -32 °C in the dark up to two months. The working standard solutions were prepared weekly, while the standard solutions for calibration curves were freshly prepared by appropriate dilution of the stock solutions with methanol.

3.2 Sludge matrix

Sludge was collected from WWTP Steinhof in the city of Braunschweig (250,000 inhabitants), Germany. Operational parameters of the WWTP are given in **Tab. 3.1**.

Table 3.1: Operational parameters of WWTP Steinhof [1]

Parameters	Description
Type of wastewater treated	Combined domestic and local industries discharges
Wastewater load (PE_{COD}/y)	350,000
Average flow rate (m^3/d)	60,000
COD (mg/L)	937 (influent); 43 (effluent)
Total solids (mg/L)	413 (influent); 9 (effluent)
Sludge production (m^3/d)	2,000 (raw); 510 (digested)
HRT (d)	21 (digestion tank)
Digestion tank operation	Thermophilic (55 °C)
Digested sludge production	510 m^3/d (2.6% d.s.)
Dewatered sludge production	464 m^3/d (28.1% d.s.)

PE: population equivalent; COD: chemical oxygen demand; HRT: hydraulic retention time

[1]Remy, 2012

The existing process of WWTP Steinhof includes mechanical (primary and secondary settling tank) and biological treatment, anaerobic sludge stabilisation in digestors, and seasonal sludge dewatering and storage on-site. The excess sludge from primary and secondary settling tanks are thickened to approximately 6.6% d.s. and subsequently digested under thermophilic conditions (54-55 °C). The digested sludge is further dewatered in a centrifuge and dried. For the study, two types of sludge were collected. The sludge, which consisted of primary and secondary sludge, was taken at the end of the primary and secondary settling tank, while the digested sludge samples were taken from the digester tank. WWTP Steinhof flow scheme and sampling points is shown in **Fig. 3.1**.

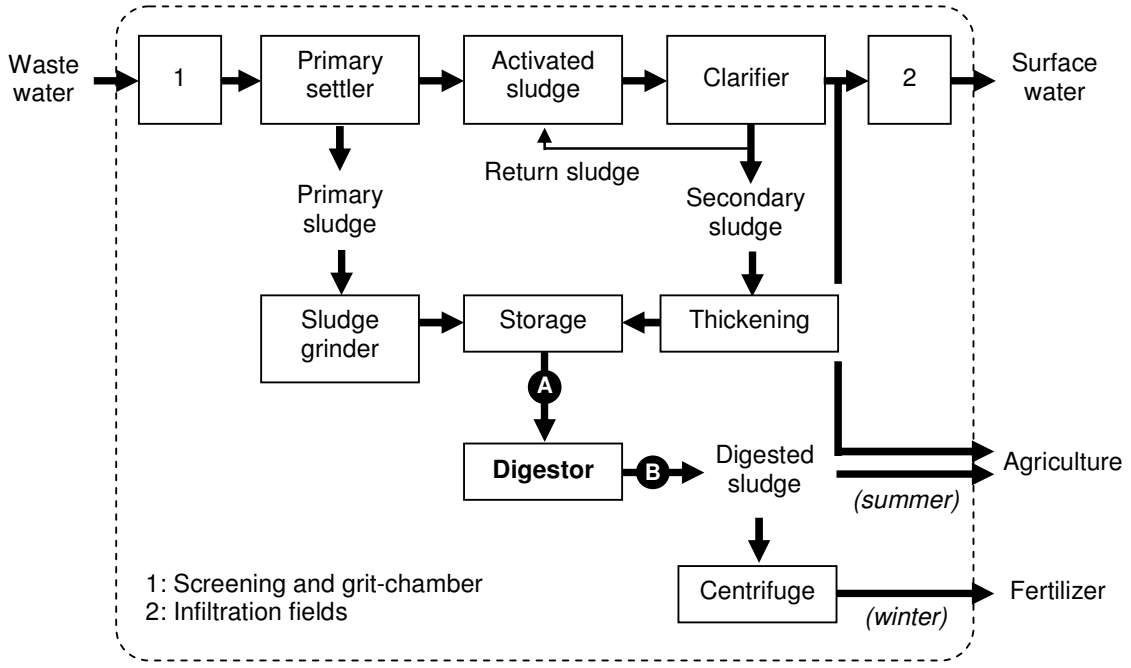


Figure 3.1: Simplified scheme of WWTP Steinhof (Remy, 2012) and collection points of sludge for the study: **A**. Raw sludge (composite of primary and secondary sludge), **B**. Digested sludge

All sludges were taken freshly before the experiment because of the impracticability of preserving the samples. The sludges were collected in PP container (5 L). Composite of primary and secondary sludge was stored at 4 °C not longer than 4 weeks while digested sludge was kept in a temperature-controlled waterbath at test temperature (54 °C) for 48 h prior to the experiments. Matrix characterizations were conducted for several parameters as summarized in **Ch. 3.3**, **Tab. 3.2**. Temperature, dry substance, pH, and redox potential (E_h) were measured as soon as possible after sludge collection.

3.3 Batch experiments

The degradation tests of BFRs were conducted in laboratory-batch systems. In these systems, the reactor was loaded with sludge matrices at the beginning of the incubation and products were taken at the end of an experimental cycle. Four different systems were set up for this study, which are: (1) anaerobic test (AN), (2) aerobic test (AE), (3) sequential anaerobic-aerobic test (AN-AE), and (4) UV/Vis-irradiation test (Light-induced, LI). For each sampling date, an individual batch was prepared and totally analyzed after the respective incubation time. System parameters were constantly monitored during the batch experiment. Details of the batch experiment set-ups are summarized in **Tab. 3.2**.

Table 3.2: Design of the batch test experiments

	AN[a]	**AE**[b]	**AN**-**AE**[c]	**LI**[d]
Type of sludge	mixture of raw[e] and digested sludge	raw[e] sludge	mixture of raw[e] and digested sludge	raw[e] sludge
Test volume	100 mL	100 mL	100 mL	-
Test duration	32 d	32 d	64 d	20 d
Test temperature	54 °C	25 °C	54 °C (**AN**), 25 °C (**AE**)	25 °C
Sampling time [d]	0, 2, 4, 8, 16, 32	0, 2, 4, 8, 16, 32	0, 2, 4, 8, 16, 32 (**AN**), 48, 64 (**AE**)	0, 1, 2, 5, 10, 20
System parameters	temperature, d.s., pH, E_h, TOC, N_{total}, NH_4-N, PO_4^{3-} (**AN**, **AE**); DO (**AE**); biogas (**AN**)			temperature

[a]**AN**: anaerobic test; [b]**AE**: aerobic test; [c]**AN**-**AE**: sequential anaerobic-aerobic test; [d]**LI**: UV/Vis-irradiation test; [e]Raw sludge: composite of primary and secondary sludge

3.3.1 Anaerobic test

40 g of a mixture of raw (a composite of primary and secondary sludge) and digested sludge (1:3 w/w) with dry substance (d.s.) of 2.6% w/w were filled into a wide-mouth amber glass bottle (capacity = 100 mL; neck i.d. = 45 mm; bottle o.d. = 50 mm; height = 70 mm). Homogenization of the sludge mixture was conducted previously by using an Ultra-Turrax homogenizer (IKA Labortechnik, Staufen, Germany) at 8000 rpm for 2×2 min. Subsequently,

a fortification of the test compounds was conducted. For that purpose, a sub portion of sludge (5 g) was filled into the glass bottle and afterwards 125 to 500 µL of standard solution was spiked directly into the sludge in order to avoid glass adsorption of the test compounds on the glass surfaces. For each sampling date, an individual flask was fortified with the respective test compound. The initial concentrations of the test compounds were in the range of 25 to 250 mg/kg sludge (d.s.) (**Tab. 3.3**). The solvent was allowed to evaporate. The rest portion of sludge was added until a final mass of 40 g. The mixture was stirred with a glass spatula to ensure uniform concentration of the test compounds in the medium. Blank experiments were performed in parallel by adding pure solvent of the same volume as in the fortification experiments. Two sets of blank samples were prepared, which were used for chemical analysis and system parameters analysis.

Table 3.3: Spiking of sludge with test compounds

Test compounds	Standard conc. [µg/µL]	Spiking vol. [µL]	Spiking level [µg]	Spiking conc. [mg/kg d.s.]
TBBPA	0.4	125	50	50
HBCD	2	125	250	250
BDE-209	0.4	125	50	50
deBDethane	0.05	500	25	25

After inoculation, the bottle was flushed with N_2 for 2 min in order to create an anaerobic conditions and then tightly capped with a rubber stopper. The stopper was equipped with a glass tube (5 mm i.d. × 10 mm) as an outlet. Incubation was conducted in a temperature-controlled open waterbath maintained at thermophilic temperature (54 °C) by means of an external heating circulator (Haake K-10, Thermo Scientific, Waltham, USA) (**Fig. 3.2**).

Incubation was run for 32 d. During the experiment, each flask was manually shaken once a day to ensure the homogeneity of the sludge. Each three days, 3 g of a fresh mixture of primary and secondary sludge (1:1, v/v) was added to the flasks in order to maintain the activity of the microorganisms which was observed by the methane production. Sampling was conducted at the defined sampling times (**Tab. 3.2**). The respective flask was collected, and the whole sludge content was used for the quantification of the respective test compound. At each sampling date, one batch was collected, and subsequent sample extraction procedure was done. Spiked and blank samples were processed in parallel under the same conditions. Exposure to light was kept as low as possible during sample preparation and analysis by covering the fume hood windows with black paper and glass

apparatus with aluminium foil. At the sampling time, redox potential (E_h), pH, and further parameters listed in **Tab. 3.2** were measured in order to assess the microbial activity of the system.

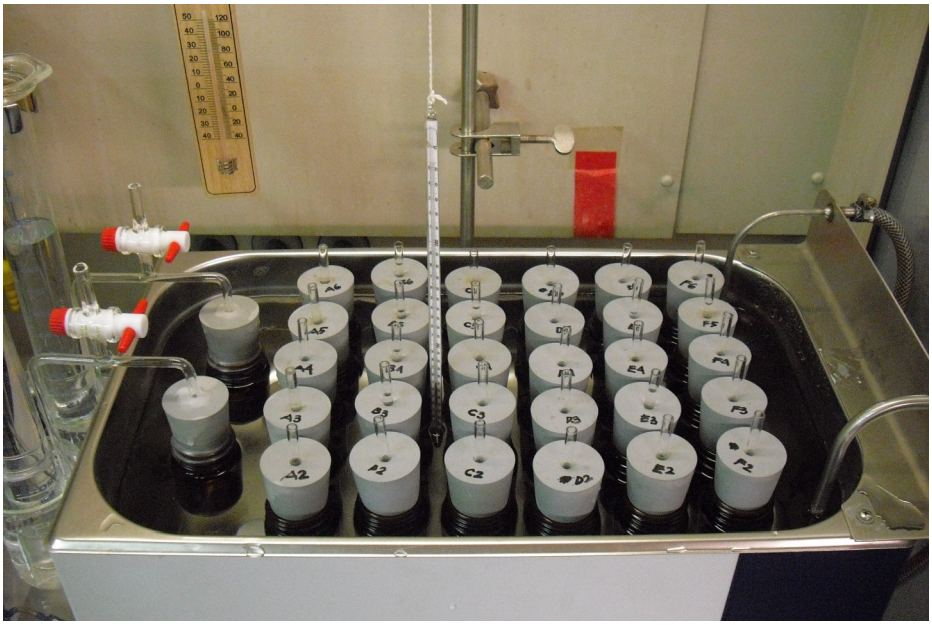


Figure 3.2: Experimental set-up of the anaerobic batch test

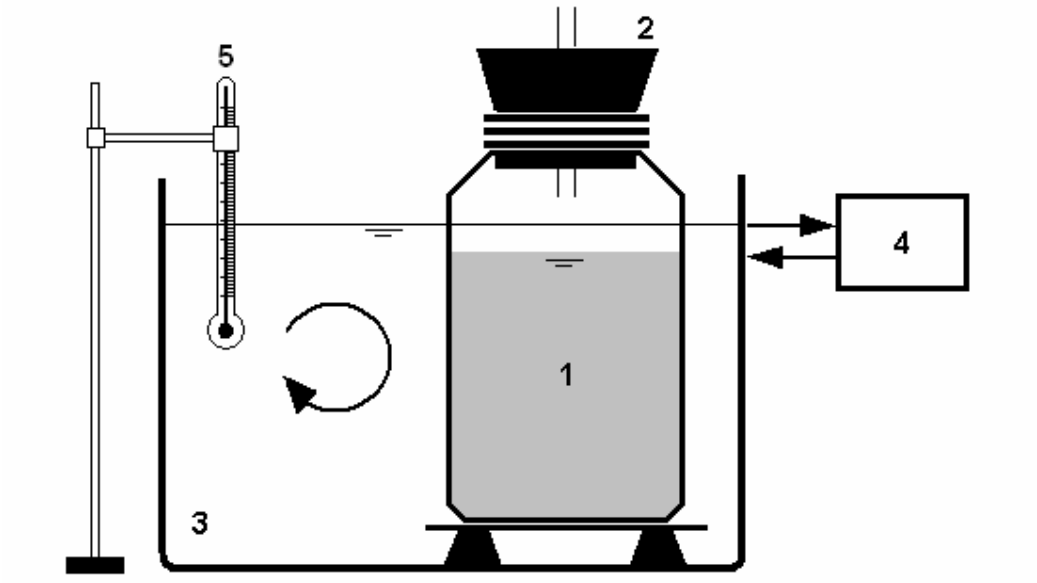


Figure 3.3: Schematic diagram of the experimental set-up of the anaerobic batch test. (1) batch reactor; (2) rubber stopper with a drilled hole and glass tube (5 mm i.d. × 10 mm); (3) temperature-controlled waterbath; (4) external heating circulator; (5) thermometer

3.3.2 Aerobic test

25 g of raw sludge (composite of primary and secondary sludge) were filled into a wide mouth amber glass bottle (capacity = 100 mL; neck i.d. = 45 mm; bottle o.d. = 50 mm; height = 70 mm). Homogenization of the sludge was conducted previously by using Ultra-Turrax homogenizer (IKA Labortechnik, Staufen, Germany) at 8000 rpm for 2×2 min. Sludge d.s. was then adjusted to ± 3% w/w by adding 15 mL Seralpur deionized water (Seral, Bansbach-Baumbach, Germany). Afterwards, the sludge was fortified with the test compounds using the same procedure as described for the anaerobic experiment (**Ch. 3.3.1**). Aerobic conditions were created by continuous aeration with a Sera air 275 R plus aquarium pump (Sera GmbH, Heinsberg, Germany) equipped with air filter and bubble diffuser stones (**Fig. 3.4**, **3.5**). Air flow was adjusted at ± 1 L/min. After installation of the diffuser, the batch reactor was covered with parafilm (Bemis, Neenah, WI, USA) in order to minimize water losses. Water level was daily adjusted with deionized water. The bottles were incubated at room temperature (25 °C) up to 32 d. The sludge was daily shaken by hand in order to maintain a homogenous mixture. Two sets of blank experiments as describe for the anaerobic experiment (**Ch. 3.3.1**), were also performed in parallel. Sampling was conducted at defined sampling times (**Tab. 3.2**), and afterwards sample extraction procedure was performed. At the sampling time, redox potential (E_h) and dissolved oxygen (DO) parameter were measured to evaluate aerobic regime of the system and further parameters listed in **Tab. 3.2** in order to assess the microbial activity of the system.

3.3.3 Anaerobic-aerobic test

The anaerobic-aerobic test was conducted in consecutive steps. The first test was done under anaerobic conditions as described in **Ch. 3.3.1.** The temperature was maintained at 54 °C and the incubation time was 32 d. As the anaerobic test was completed, the sample was subsequently transferred to the second system. The second test was done under aerobic conditions by the same way as described in **Ch. 3.3.2**. The aerobic reactor worked at room temperature and the incubation time was 32 d. Thus, total incubation time for anaerobic-aerobic test was 64 d. Two sets blank experiments were also performed in parallel. Sampling activity was done as described in **Tab. 3.2** and parameters listed in **Tab. 3.2** were measured in order to assess the microbial activity of the system.

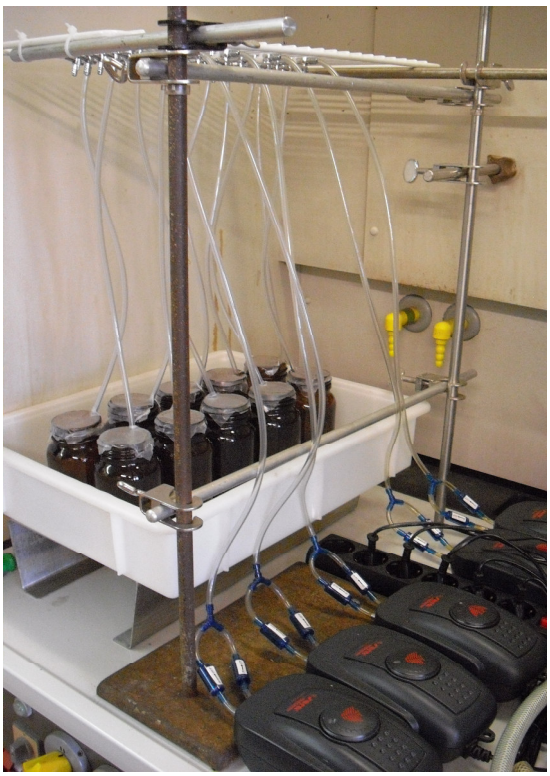


Figure 3.4: Experimental set-up of the aerobic batch test

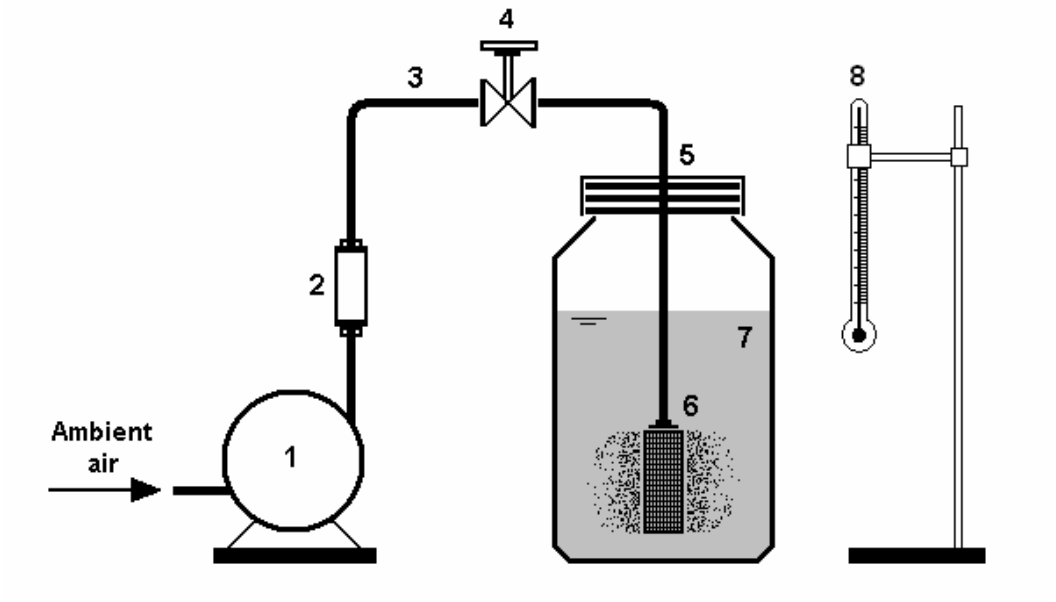


Figure 3.5: Schematic diagram of the experimental set-up of the aerobic batch test. (1) air pump; (2) air filter; (3) plastic tubing (5 mm i.d.); (4) gas valve; (5) parafilm; (6) diffuser; (7) batch reactor; (8) thermometer

3.3.4 UV/Vis-irradiation test

Studies of light-induced dissipation during drying of sludge under sunlight were conducted with an artificial UV/Vis light source. An irradiation apparatus (Heraeus, Hanau, Germany) (**Fig. 3.6**) was equipped with a mercury (Hg) medium-pressure lamp (Schott, Mainz, Germany). The lamp is covered with an outer cooling cylinder from borosilicate glass which was filled with a mixture of water and ethylene glycol (1:1 v/v) and connected to Haake K-10 external cooling circulator (Thermo Scientific, Waltham, USA). Due to the borosilicate glass cylinder, the lamp had a wavelength cut-off of 290 nm. The power supply of the apparatus was connected to a timer in order to regulate defined irradiation periods. The test was done inside paper-covered fume hood to avoid exposure of external light.

20 g of raw sludge (composite of primary and secondary sludge) was filled into a porcelain dish (130 mm o.d.). Subsequently, the sludge was fortified with the test compounds as described for the anaerobic experiment (**Ch. 3.3.1**). The spiked sludge was spread over the surface of the disk to set a uniform sludge layer. Prior the experiment, the samples were kept in the dark allowing the solvent to evaporate. Six samples were placed under the Hg-lamp in a distance of 150 mm to the sludge surface. The position of the dishes was exchanged every 4 h to ensure an equal of light exposure to each sample. The intensity of the lamp radiation during the experimental period was measured by using a Voltcraft MS-1300 luxmeter (Conrad Electronic SE, Germany). In order to simulate day-night cycles, the light was switched on for 12 h/d. The surface temperature of the sample was regularly measured.

A dark experiment was performed simultaneously in a Heraeus drying oven (Heraeus Instruments GmbH, Hanau, Germany) to maintain a constant temperature of 30 °C, equal to the temperature of the sludge surface during irradiation experiment. Oven temperature was continuously monitored by using a Thermo Alert 07/06 temperature sensor (Conrad Electronic, Hirschau, Germany). Dark and light experiments run for 20 d. At the defined sampling times (**Tab. 3.2**), the test was terminated by removing the sample from the light exposure. Afterwards, irradiated and dark samples were extracted at the same time under the same conditions.

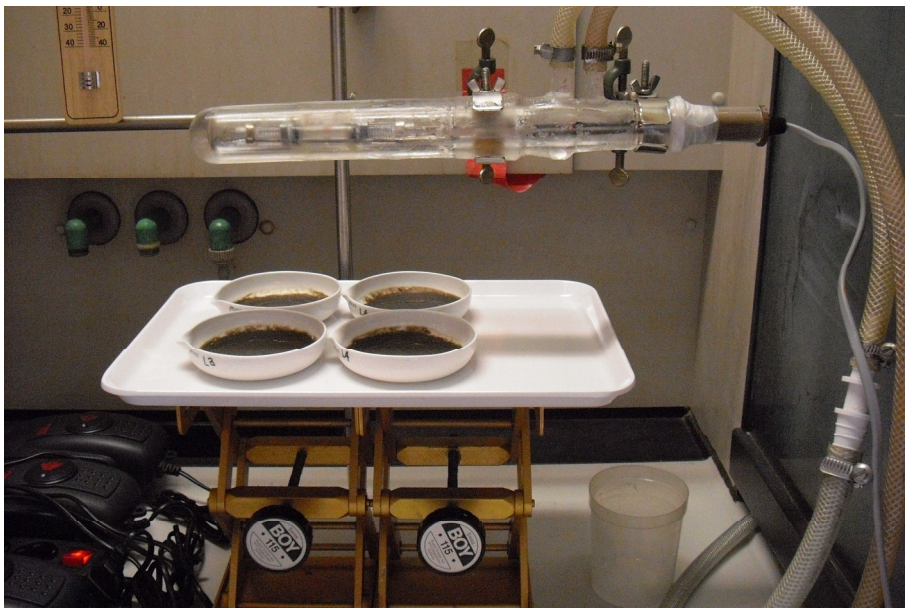


Figure 3.6: Experimental set-ups for UV/Vis-irradiation test

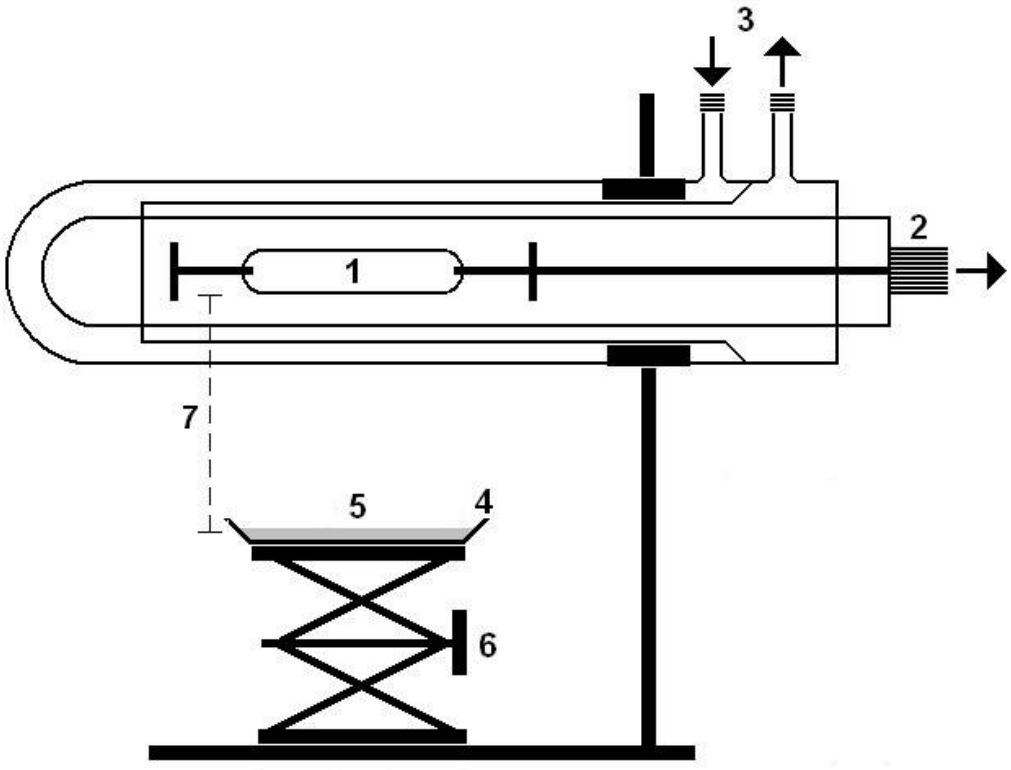


Figure 3.7: Schematic diagram of the experimental set-up of the UV/Vis-irradiation test. (1) Hg-lamp (cut-off: λ = 290 nm); (2) power supply connected to a timer; (3) cooling device; (4) porcelain dish (130 mm o.d.); (5) sludge sample; (6) lifting platform; (7) irradiation distance (150 mm)

3.4 Process parameters of the batch tests

Process parameters were constantly monitored during the batch experiment. Parameters measurement was based on the established methods (Kreuzig et al., 2007).

3.4.1 Dry substance (d.s.)

For the determination of the dry substance (d.s.), an ultra-X infrared heater (Gronert, Germany) was used. For this procedure, 3 to 5 g of the homogenized sludge was equally distributed in an evaporating dish on the weighing scale. Afterwards, the infrared heater was switched on, and water evaporation was performed until mass constancy. The dry substance content was calculated according to following equation:

$$\text{d.s.}\,\% = \frac{m_b}{m_a} \times 100$$

d.s. = dry substance [%]
m_a = initial mass [g]
m_b = output mass [g]

3.4.2 pH

The pH value was measured directly in the homogenized sludge by means of a pH meter Multical 535 GLP (WTW, Weilheim, Germany) equipped with SenTix61 glass electrode (pH 0-14, 0-100 °C, 3 mol/L KCl) (WTW, Weilheim, Germany). The pH meter was calibrated before each measurement using three different buffer solutions (pH 4.0, 7.0, 9.2), which were freshly prepared every month.

3.4.3 Redox potential (E_h)

Multical 535 GLP (WTW, Weilheim, Germany) equipped with InLab 501 combination redox electrode (Mettler-Toledo, Greifensee, Switzerland) was used to measure the sludge redox potential (E_h) which was calculated according to the following equation:

$$E_h = E_m + E_r$$

E_h = electrode potential referred to the standard hydrogen electrode [mV]

E_m = measured oxido-reduction potential [mV]
E_r = potential of the reference electrode (Ag/AgCl) at the measurement temperature [mV]

Prior to measurement, the redox electrode was standardized by determining its response (E_m) in a redox buffer solution. The reading was recorded when the difference between successive measurements at 5 min is ≤ 5 mV.

3.4.4 Dissolved oxygen (DO)

Oxygen concentration was measured with a DO-meter Oxi 340i (WTW, Weilheim, Germany) connected with a CellOx 325 DO electrode (WTW, Weilheim, Germany). The electrode was immersed in the sludge sample until a stable reading was obtained. Prior the measurement, the electrode calibration was performed in water vapor-saturated air condition by using OxiCal-SL air calibration vessel. DO value is expressed as mg O_2/kg sample.

3.4.5 Total organic carbon (TOC)

Sample preparation. Sludge samples were treated with an excess of 4 M HCl for the removal of carbonates. The excess HCl was removed by heating the sample on a hot plate at 100 °C. Afterwards, the samples were dried overnight in an oven at 105 °C. After cooling, the samples were ground using mortar and pestle, and then kept in a desiccator until TOC analysis.

Sample analysis. TOC was determined by means of Dohrmann DC-90 organic carbon analyzer (Dohrmann, Santa Clara, CA, USA). For this purpose, 3 mg of treated samples were combusted in an oxygen stream at 900 °C. The released carbon dioxide was subsequently detected by a non-dispersive infrared detector. The TOC amount was calculated based on an external standardization. The calibration curves were recorded using a mixture of oxalic acid dehydrate and aluminum oxide (Merck, Darmstadt, Germany) in a ratio of 1:9 (w/w), respectively. Finally, the results were expressed in % of dry substance according to the following equation:

$$TOC = \frac{m_c \times f}{m_a} \times 100$$

TOC = total organic carbon [%]
m_a = initial weight [µg]

m_c = amount of carbon [µg]

f = dilution factor

3.4.6 Total Kjeldahl Nitrogen (TKN)

Sample digestion. 3 g of the homogenized sludge was added to the digestion vessel (2 replicates). 1 Kjeldahl tablet (Missouri catalyst, 5 g) (Merck, Darmstadt, Germany), 10 mL H_2SO_4 sulfuric acid (≥ 95%) (VWR, Fontenay-sous-Bois, France), and 3 boiling stones were added to each vessel. Fortified samples were prepared by adding 50 mg phenylalanine (Sigma-Aldrich, Steinheim, Germany) in order to determine the recovery rate of the method. Blank samples were also prepared which contained a Kjeldahl tablet and H_2SO_4 only. The digestion process was performed using a block digester system (Digestor 430, Büchi, Switzerland) inside the fumehood. The instrument was programmed as follows: level 3/10 (225 °C) for 30 min, level 6/10 (500 °C) for 30 min, and then level 10/10 (770 °C) for 30 min or until the sample solution was clear and the white cloud inside the vessels disappeared. At the end of the digestion, all vessels were removed from the digestion unit and left in the fumehood to cool down for 30 min.

Sample analysis. After the digestion, the vessel was inserted into the distillation unit (B-323 Distillation Unit, Büchi, Switzerland). 50 mL of boric acid (20 g/L) (Merck, Darmstadt, Germany) and 200 µL mixed indicator 5 (Merck, Darmstadt, Germany) were prepared in a 250 mL Erlenmeyer flask for each digestion vessel. The flask was placed under the condenser of the distillation apparatus. The following parameters were programmed: addition of 30 mL of water and 70 mL of 3 M NaOH (Carl Roth, Karlsruhe, Germany), 10 min distillation time with 30% steam power. At the end of distillation, the distillate were titrated with 0.1 M HCl from green to weakly pink. The same procedure was performed with the blank sample. The consumption of HCl was notified and the TKN in the sample was calculated based on the following equation:

$$TKN = \frac{(V_1 - V_0) \times c \times M_N}{m}$$

TKN = total Kjeldahl nitrogen [g N/kg]

V_0 = volume of HCl used in the blank test [mL]

V_1 = volume of HCl used in the titration of the blank sample [mL]

m = mass of the sludge sample [g]

c = concentration of HCl [mol/L]

M_N = molar mass of N [g/mol]

3.4.7 Ammonia

2 g of the homogenized sludge was transferred into a digestion vessel (2 replicates). The digestion vessel was inserted to the distillation unit (B-323 Distillation Unit, Büchi, Switzerland) and sample distillation was performed as described for Total Kjeldahl Nitrogen. The same procedure was performed with a blank sample. The consumption of HCl was notified and the ammonium nitrogen (NH_4-N) concentration in the sample was calculated based on following equation:

$$NH_4 - N = \frac{(V_1 - V_0) \times c \times M_N}{m}$$

NH_4-N = mass fraction of ammonia [g NH_4/kg]
V_0 = volume of HCl used in the blank test [mL]
V_1 = volume of HCl used in the titration of the blank sample [mL]
m = mass of the sludge sample [g]
c = concentration of HCl [mol/L]
M_N = molar mass of N [g/mol]

3.4.8 Nitrate, nitrite, and inorganic salts

Sample preparation. 5 g of sludge sample was centrifuged using a Megafuge 1.0 (Heraeus Sepatech GmbH, Hanau, Germany) at 3000 rpm for 10 minutes. The liquid phase was gently poured out and then was filtered through a Chromafil PET 0.45 µm syringe filter (Macherey-Nagel, Düren, Germany).

Sample analysis. The analysis of soluble nitrite, nitrate, and inorganic salts (phosphate, chloride, phosphate) was performed using a 690 Ion Chromatograph (Metrohm, Switzerland) in combination with a 697 IC Pump and 698 Autosampler. Chromatographic conditions were as follows: Metrosep Anion Dual 2 (75 mm × 4.6 mm i.d.) column with a mobile phase of Na_2CO_3 (1.30 mM) and $NaHCO_3$ (2.00 mM) in pure water, a flow rate of 0.8 mL/min, a column oven temperature of 35 °C, a pressure of 35 bar, and a conductivity detector. The injection volume was 200 µL. The concentration was determined by establishing a calibration curve with a multi-element standard solution (Merck, Darmstadt, Germany) at the concentration range of 1-10 mg/L.

3.4.9 Biogas

Biogas production was measured by the principle of liquid displacement in a height gasometer (**Fig. 3.8**). For that matter, biogas was introduced into a volumetric cylinder filled with acidified water. Acidified distilled water (pH = 3) was used in order to avoid the solution of CO_2 in the water phase. Gas volume was calculated from the change of the height of the water level in the cylinder.

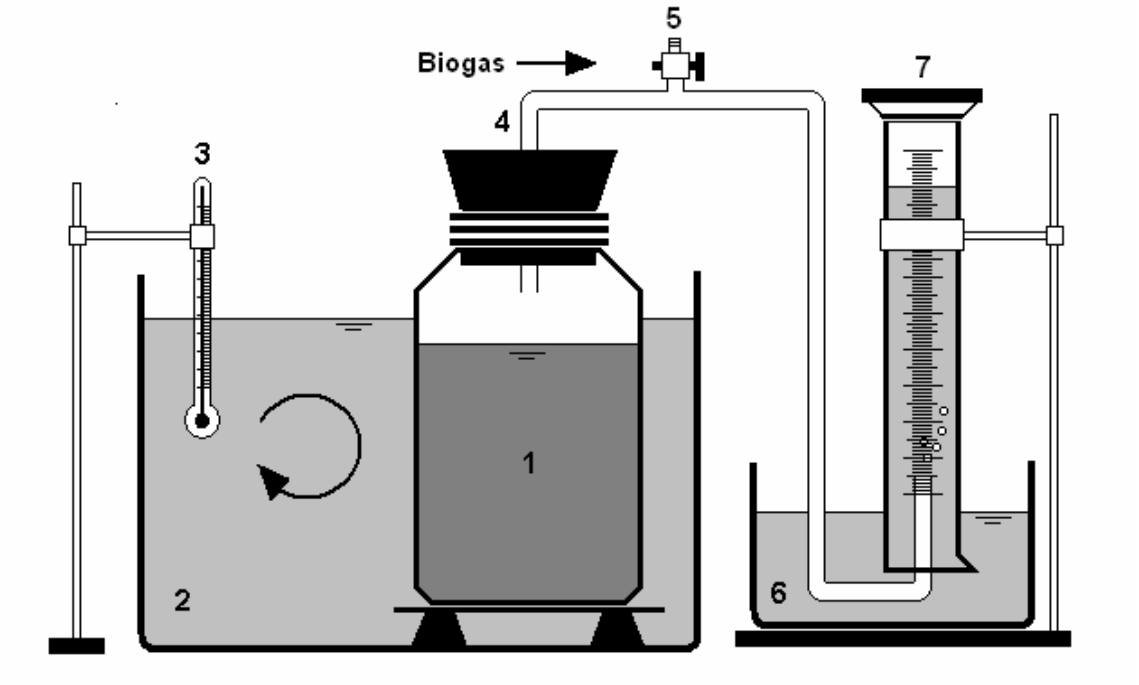


Figure 3.8: Schematic diagram of biogas measurement apparatus using a height gasometer: (1) batch reactor; (2) temperature-controlled waterbath; (3) thermometer; (4) glass tube (450 mm × 5 mm i.d.); (5) gas valve; (6) beaker filled with acidified distilled water (pH = 3); (7) volumetric cylinder filled with acidified distilled water (pH = 3)

Semi-quantitative determination of methane in the produced biogas was performed. When biogas reached the maximum volume in the height gasometer, a rubber stopcock equipped with a gas valve was fixed on the mouth of the cylinder. This procedure was done under the water to avoid biogas loss. The cylinder was then turned to right-side up position, and parafilm was sealed to the rubber stopcock. A sub-volume of the collected biogas was taken by a 20 mL sampling syringe. The syringe was connected with an elastic tube to the measurement cylinder by a gas valve (**Fig. 3.9**). Subsequently, 150 µL of gas sample was taken by using a gas-tight syringe (Hamilton, Bonaduz, Switzerland) from the rubber hose of the sampling syringe.

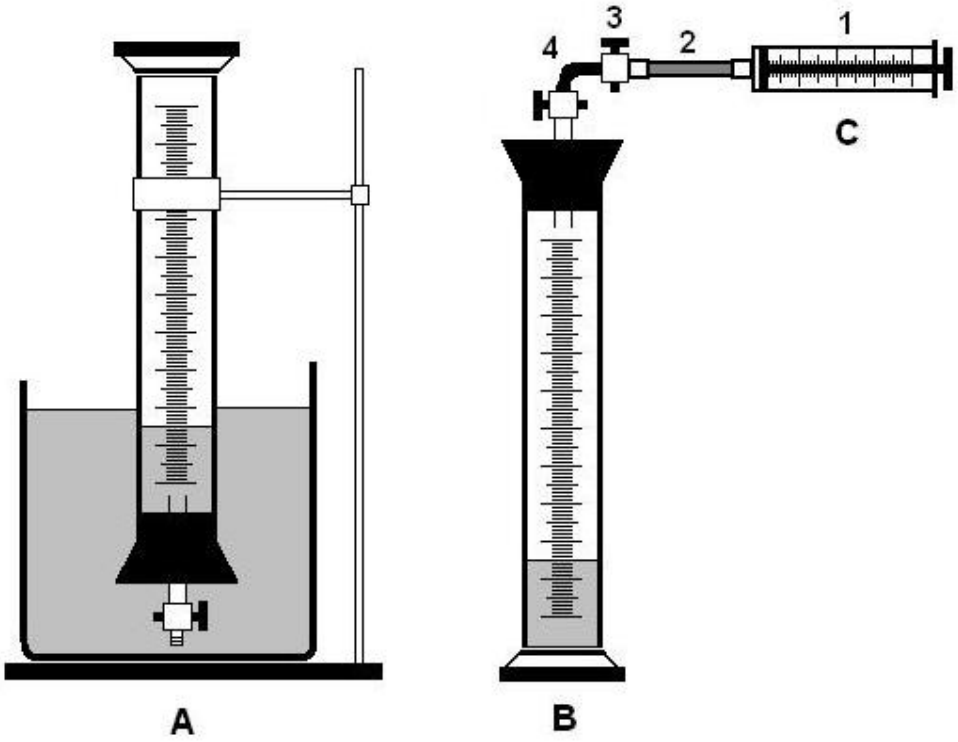


Figure 3.9: Biogas sampling technique from height gasometer. **A.** Volumetric cylinder contains maximum volume of biogas fitted with rubber stopcock equipped with gas valve. **B.** Biogas sampling by using sampling syringe. **C.** Sampling syringe: (1) gas tight syringe, (2) rubber hose, (3) gas valve, (4) elastic tube connector

Determination of methane was carried out with a gas chromatography coupled with thermal conductivity detector (GC/TCD) (Focus, Thermo Scientific, Milan, Italy). For the calibration, methane and nitrogen gas mixtures in the ratios 5 % v/v CH_4 and 95 % v/v N_2, 35 % v/v CH_4 and 65 % v/v N_2 and 65 % v/v CH_4 and 35 % v/v N_2 were prepared and measured. The obtained calibration curve was sufficiently linear with a coefficient of determination of R^2 = 0.999.

GC parameters for gas analysis were as follows: the carrier gas was helium, and a packed column, Shin carboxylic ST 100/120 (2 m length, 1 mm i.d. and 1/16" o.d.) (Resteck, Bellefonte, Pennsylvania) with a temperature gradient was used. The starting temperature of the GC oven was 40 °C for 3.7 min. Then it was heated with a heating rate of 20 °C min^{-1} to 170 °C and held for another 4 min. The total run time time was 14 min.

3.5 Analytical methods for BFRs

An overview of the finally applied analytical method for the test compounds in sludge is presented in **Fig. 3.10**.

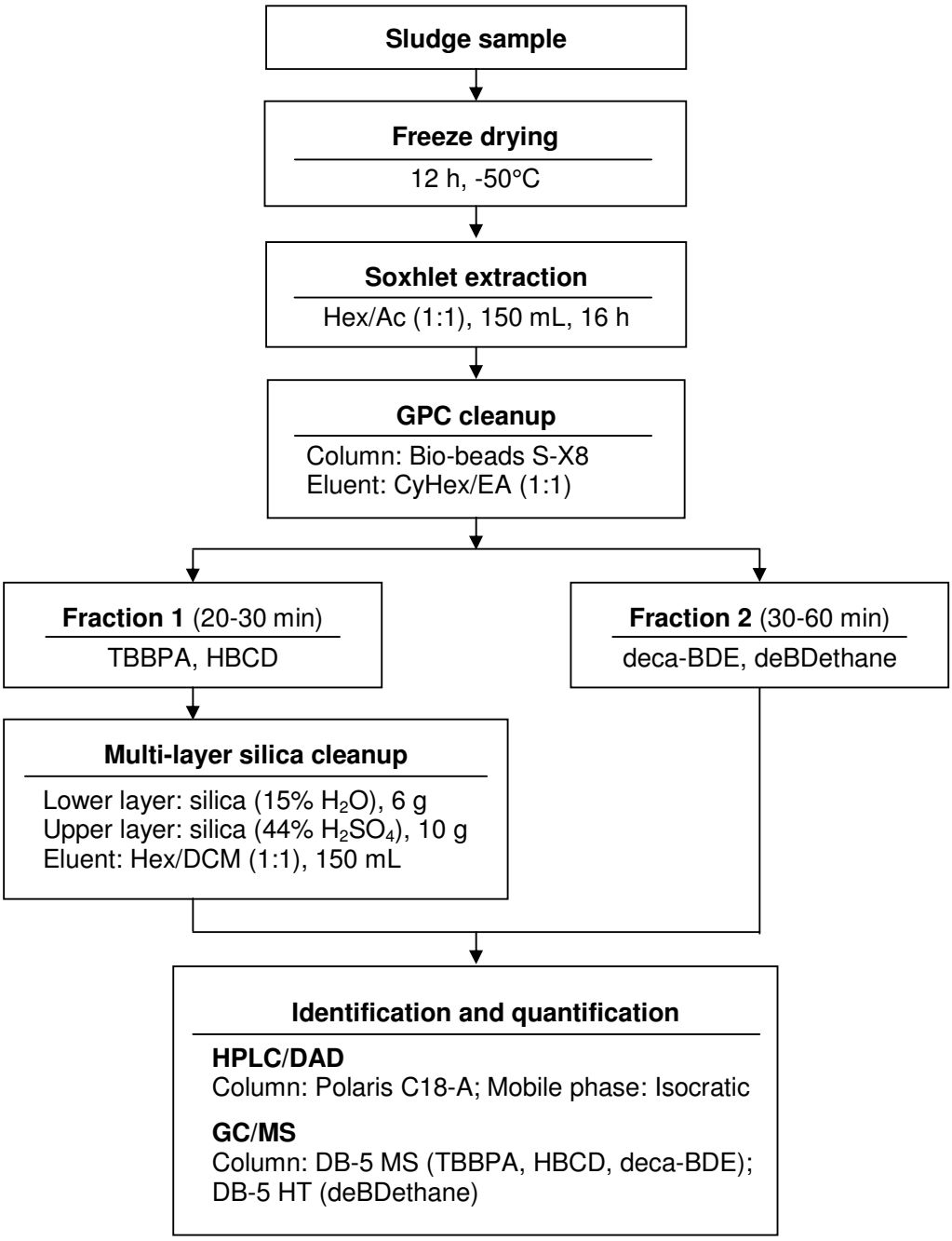


Figure 3.10: Schematic diagram of the analytical method for BFRs in sludge matrices. Hex: n-hexane; Ac: acetone; CyHex: cyclohexane; DCM: dichlormethane; MeOH: methanol; IPA: isopropanol

3.5.1 Extraction

Wet sludge samples were cooled in the refrigerator at -32 °C overnight and were further cooled down with liquid nitrogen at the next day. Freeze-drying (Lyolab A, LSL Secfroid, Lausanne, Switzerland) was then performed at -50 °C and under low-pressure conditions (0.05 mbar) for 12 h. Lyophilized sludge was mixed with about 10 g of anhydrous Na_2SO_4 (Roth, Karlsruhe, Germany) until a free-flowing sludge matrix was obtained and then transferred into 33×80 mm single-layer cellulose extraction thimbles (Schleicher & Schuell GmbH, Dassel, Germany). For TBBPA samples, 200 µL sulfuric acid (≥95%) (VWR, Fontenay-sous-Bois, France) was also added. Soxhlet extraction was performed in a 100 mL Soxhlet apparatus with 150 mL binary solvent mixtures n-hexane/acetone (1:1 v/v). Extraction time was 16 h at a rate of 4-5 cycles per h. The extract was volume-reduced in a rotary evaporator (Labo-Rota C-311, Resona Technics, Switzerland) and solvent-exchanged to 5 mL ethyl acetate and cyclohexane (1:1 v/v) for GPC cleanup.

3.5.2 Cleanup

GPC

A Gilson GPC unit (Abimed, Langenfeld, Germany) consisting of an isocratic HPLC-pump (model 302), an auto-sampler injector (model 231), a dilutor (model 401), a fraction collector (model 201), and a chromatography column of glass (59 cm × 2.9 cm i.d.) packed with 38 cm of polystyrene-divinylbenzene adsorbent Bio-Beads SX-8 (200-400 mesh) (Bio-Rad Labs., Richmond, USA) were used. The eluting solvent was a mixture of ethyl acetate and cyclohexane (1:1 v/v). The flow rate was set to 5.0 mL/min and the injection volume was 4.0 mL. 5 mL of spiked sludge extract was micro-filtered using a syringe filter (Chromafil PET 20/25, Macherey-Nagel, Detlef Lambrecht, Germany) and transferred to a GPC vial. 4 mL aliquot was injected into the GPC apparatus and two fractions were collected, fraction 1 (50 mL, from 20 to 30 min) containing TBBPA and HBCD and fraction 2 (150 mL, from 30 to 60 min) containing BDE-209 and deBDethane. The collected fractions were volume reduced and redissolved in n-hexane to a final volume of 1 mL.

Multi-layer silica gel column chromatography (fraction 1)

The silica gel was activated at 160 °C overnight. Afterwards, one part was deactivated 15% by adding deionized water, and another part was impregnated with 44% H_2SO_4. In order to ensure homogeneous distribution of the water or H_2SO_4, the mixture was shaken on a horizontal shaker type 3020 (GLF, Burgwedel, Germany) at 200 rpm for 1 h. The chromatographic column (300 mm × 20 mm i.d.) was packed first with 10 g silica gel (15%

H_2O), followed by 6 g silica gel (44% H_2SO_4), and finally 1 g of oven-dried anhydrous Na_2SO_4. The column was conditioned with 50 mL of n-hexane. Then, the GPC extract was transferred on the multi-layer silica gel column and was eluted with 50 mL n-hexane/DCM (1:1 v/v). The flow rate was adjusted to 2 mL/min. Finally, the collected fraction was rotary evaporated and reconstituted in methanol to a final volume of 1 mL.

Removal of sulphur

Removal of elemental sulphur from sludge extracts, which disturb during GC/MS analysis, was performed by a GPC cleanup. During GPC, sulphur was eluted as discarded eluate for first 20 min (100 mL) before the collection of fractions contain test compounds. The fractions collection begins from 20 to 30 min (50 mL) as fraction 1, and from 30 to 60 min (150 mL) as fraction 2. An efficient sulphur removal was obtained for fraction 2, containing BDE-209 and deBDethane. For fraction 1, only partial removal of sulphur was achieved. However, during GC/MS analysis the peak of sulphur was not overlapped with peak of the test compounds, which were TBBPA and HBCD.

Derivatization of TBBPA (fraction 1)

A derivatisation step was needed for TBBPA before GC-MS analysis. TBBPA was collected in the fraction 1 during GPC cleanup (20 to 30 min). The extract in 10 ml of n-hexane was derivatised with 500 µL acetic acid anhydride and pyridine (1:1 v/v) at 60 °C for 30 minutes. After derivatisation, the organic phase was extracted with 10 ml of water to remove the excess of acetic acid anhydride and pyridine.

3.5.3 Quantification

HPLC/DAD analysis

The test compounds analyses were performed by using HPLC/DAD. Agilent 1100 series (Agilent Technologies, Palo Alto, CA, USA) was used, which was equipped with vacuum degasser, binary pump, column oven, and autosampler. The HPLC conditions were as follows: Varian Polaris C18-A reversed-phase column (150 × 4.6 mm, 3 µm particle size) (Agilent Technologies, Palo Alto, CA, USA) was used with a column temperature set to 40 °C for deBDethane and 20 °C for the other compounds. Different isocratic mobile phases were used (**Tab. 3.4**). The flow rate was 0.8 mL/min, the injection volume 10 µL, and the detection wavelength set to 210 and 225 nm.

Table 3.4: HPLC/DAD isocratic mobile phase

Test compounds	Mobile phase	Column temperature [°C]	Detector wavelength [nm]
TBBPA	MeOH/H_2O (75:25, v/v), pH = 4[a]	20	210
HBCD	MeOH/H_2O (85:15 v/v)	20	210
BDE-209	MeOH	20	225
deBDethane	MeOH/IPA (50:50 v/v)	40	225

[a]adjusted by the addition of formic acid (>96%) (Roth, Karlsruhe, Germany)

GC/MS analysis

The identification of degradation products was performed using GC/MS. Agilent GC 6890 series (Agilent Technologies, Palo Alto, CA, USA) was used which was equipped with HP 7683 series injector, and Agilent 5975C inert MSD with triple-axis detector. The GC conditions were set as follows: helium with a flow rate of 1 mL/min was used as the mobile phase. The used GC columns and temperature programs are summarized in **Tab. 3.5**. For MS, the ionization voltage (EI) was set at 70 eV and the electron multiplier voltage (EMV) at 1518 V. Ion source temperatures was set at 230 °C. The GC/MS interface temperature was set at 305 °C (method 1 and 2) or 320 °C (method 3). A pulsed splitless injection mode was used, and the injector temperature was set at 300 °C. The injection volume was 1 µL (method 1) and 2 µL (method 2 and 3). The mass spectra were scanned from *m/z* 40-1000. The compounds were identified by matching peak retention times (t_R) with those obtained from authentic standards and by comparison of their recorded mass spectra with those of the spectra library.

Table 3.5: GC/MS temperature programs

Test compounds	Type of capillary column	Temperature program
TBBPA, HBCD	DB-5 MS[a] (30 m × 0.25 mm i.d., 0.25 µm film thickness)	**Method 1**: 110 °C (2 min) to 200 °C at rate of 20 °C/min, to 305 °C at 15 °C/min (20 min); total run time = 33.50 min
BDE-209	DB-5 MS[a] (30 m × 0.25 mm i.d., 0.25 µm film thickness)	**Method 2**: 110 °C (2 min) to 305 °C at 12.5 °C/min (40 min); total run time = 57.60 min

Table 3.5: Continued

Test compounds	Type of capillary column	Temperature program
deBDethane	DB-5 HT[a] (30 m × 0.25 mm i.d., 0.10 µm film thickness)	**Method 3**: 110 °C (2 min) to 325 °C at 12.5 °C/min (22.5 min); total run time = 41.70 min

[a]purchased from J&W Scientific, Folsom, CA, USA

3.5.4 Validation of the analytical method

The analytical method was validated by the determination of linearity, sensitivity, accuracy, and precision. The quantification was performed by external calibration. The peak areas were fitted by linear regression and correlation coefficients (R^2) = ≥0.995 were accepted. Precision and accuracy were evaluated by performing fortification experiments for all test compounds at concentrations of 5 and 50 mg/kg d.s. Recovery rates were calculated by comparing the peak areas from samples spiked with a mixture of test compounds before extraction with the peak area obtained from the standard solutions. Recovery rates were expressed as a percentage. The precision was expressed as relative standard deviation (RSD), where 20% was the maximum acceptable value, according to the criteria of the EU (SANCO/2007/3131). The limit of detection (LOD) and the limit of quantification (LOQ) were determined by signal to noise ratio (S/N) method of calibration standards. LOD is defined as the signal to noise ratio (S/N) equal to 3 and LOQ equal to 10.

3.5.5 Calculation of dissipation kinetics

For the assessment of the kinetics, dissipation of the test compounds were assumed to follow a first-order kinetic model, as suggested by many authors (Davis et al., 2005, 2006; Gerecke et al., 2006; Matthies et a., 2008; Nyholm, et al., 2010). This approach is commonly used when chemicals are present at low concentrations in the environment (mg/kg). Pseudo-first-order kinetics was assumed when there are negligible changes in biomass levels that occur as a result of growth (Larson et al., 2000). First, concentrations of the test compounds were normalized to the initial concentration and examined versus time. From a plot of the natural logarithm of the concentration as a function of time, kinetics of the test compounds loss was determined. The first-order dissipation rate constant, k, thus was calculated from Eq. 3.1.

$$k = \frac{\ln\left[\frac{C_0}{C_t}\right]}{t} \quad (3.1)$$

k = dissipation rate constant

C_0 = the initial compound concentration at the time t = 0

C_t = compound concentration at the time t

t = time

Regression analysis was used to determine the rate constant for each set of compounds by the least-squares method. Subsequently, the time required for 50% dissipation (DT_{50}) can be determined using the Eq. 3.2. A fast dissipation is described by a high k-value and low DT_{50} value.

$$DT_{50} = \frac{\ln\left[\frac{C_0}{\frac{C_0}{2}}\right]}{k} = \frac{\ln 2}{k} \quad (3.1)$$

DT_{50} = the time required for 50% dissipation

k = dissipation rate constant

C_0 = the initial compound concentration at the time t = 0

4. Results and Discussion

4.1 Method development for BFRs analysis

In order to investigate the BFR's fate in different batch tests, first an analytical method was developed. The starting point of the method development was to establish and to validate the method for quantification of the test compounds with HPLC/DAD. This step was followed by the selection and optimization of the cleanup method using spiked sludge extracts without optimizing the extraction procedure. Finally, the selection and optimization of extraction method was carried out. GC/MS methods were worked out in order to detect the degradation product during the batch tests.

4.1.1 Identification and quantification

In this step, an HPLC-DAD method for all four test compounds was developed as the basis for the further method development. Several reversed-phase (RP) columns with different dimensions were compared. In general, Zorbax SB C-18 column (Agilent Technologies, Waldbronn, Germany) (150 × 3 mm; 3.5 µm particle size) and Polaris C-18A (Varian Inc., CA, USA) (150 × 4.6 mm; 3 µm particle size) gave the best results. Trials using a column with larger length and particle size, i.e. Ultrasphere ODS C-18 (250 × 4.6 mm; 5 µm particle size) and Lichrospher 100 RP-18 (250 × 4 mm; 5 µm particle size) produced very long elution times for some test compounds. For example, the t_R of 29.27 and 33.05 min for deBDethane were obtained under isocratic elution with methanol at a flow rate 1 mL/min in Ultrasphere ODS C-18 and Lichrospher 100 RP-18, respectively. Finally, endcapped Polaris C-18A was chosen because it provided better peak shape of the test compounds than the non-endcapped Zorbax SB C-18.

Subsequently, the composition of the mobile phase was optimized. In the RP system, the high brominated compounds, BDE-209 and deBDethane, have very long retention time due to their unpolar character. In order to reach a short elution time for deBDethane, which was the most retained compound, a high elution power of the mobile phase was needed. Furthermore, the early-eluting polar substances, TBBPA and HBCD, led to problems through interferences with also early-eluting matrix peaks. A mixture of methanol and water was selected as starting point for the method development. Chromatographic resolution of TBBPA and HBCD was increased by increasing the water content in the mobile phase up to 10% v/v.

Starting with this composition of mobile phase A, a gradient elution was applied: after 6 min elution time with methanol/water (90:10 v/v), the methanol was increased to 100% in 2 min and held at this composition for the rest of the run. However, the elution power of 100% methanol was not sufficient to prevent peak tailing for the latest eluting compound, deBDethane. Thus 10% v/v of THF was added to mobile phase in order to enhance the elution power. By this modification, the retention time was shortened (from 16.62 to 12.74 min), and the peak shape of deBDethane was optimized. In order to further improve the peak shape of deBDethane, the column temperature was adjusted at 40 °C.

For DAD detection, the optimum wavelengths (λ_{max}) were selected from the UV spectra of the test compounds in the chromatogram (**Fig. 4.1**). The λ_{max} of the test compounds varied, depending on the chemical structure of the respective compounds. Due to its aromatics rings, TBBPA showed a maximum UV absorption at 205 nm with a comparably high detection response (220 mAU). In contrast, considering the lack of aromatic rings of HBCD, only a weak UV response (20 mAU) was detected at 205 nm at a 5 fold higher concentration than TBBPA. DAD detector wavelength of 210 nm was chosen during analysis for both compounds. This wavelength produced a lower DAD detector response. However, in parallel it reduced disturbance from coextracted compounds.

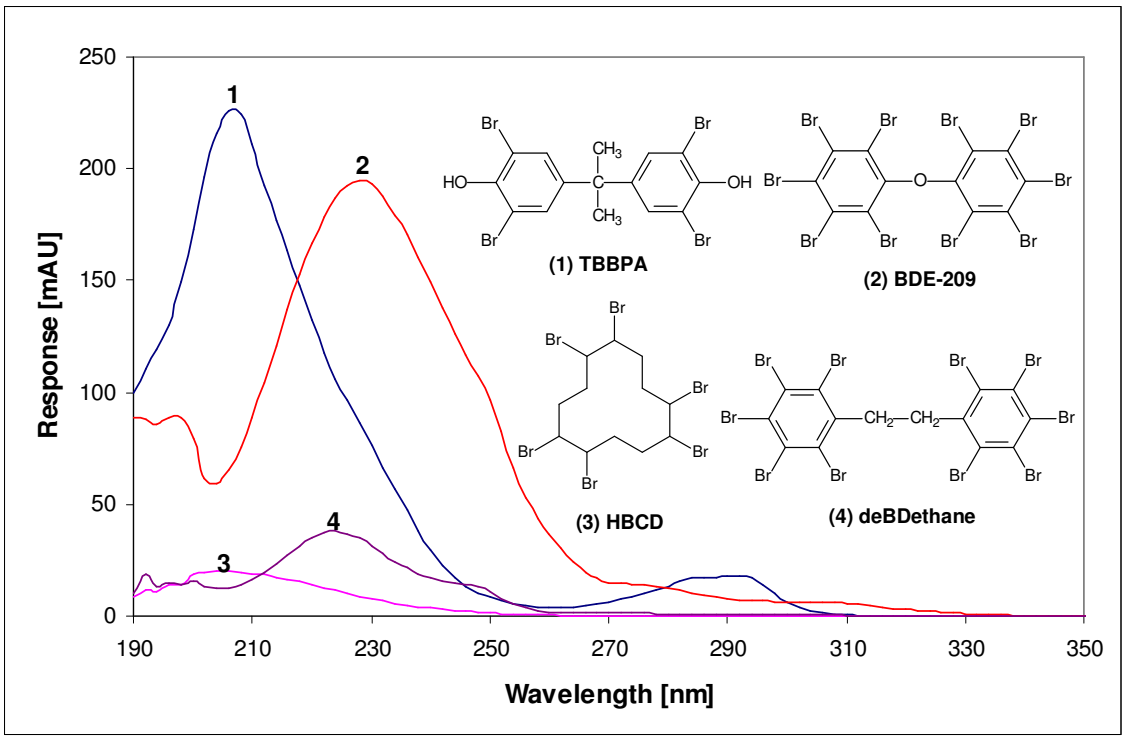


Figure 4.1: UV spectra of the test compounds: (1) TBBPA (50 ng/µL), (2) BDE-209 (50 ng/µL), (3) HBCD (250 ng/µL), and (4) deBDethane (25 ng/µL)

BDE-209 and deBDethane had a similar maximum UV absorption at 230 and 225 nm, respectively. However, deBDethane showed a lower detection response than BDE-209 (by factor 4). This factor can be explained as the concentration of deBDethane was only the half. The further reduction of intensity might be caused by the presence of the ethane bridge between its aromatic rings which reduces the chromophoric system. For analysis, detector wavelength of 225 nm was chosen for both compounds.

The optimized HPLC/DAD method showed a good separation of all peaks and optimized peak shape (**Tab. 4.1**). However for deBDethane, asymmetry factor of 1 was slightly <1, indicating a slightly fronting peak.

Table 4.1: Peak parameters using the HPLC/DAD method optimized for the test compounds

Test compounds	t_R [min]	Rs [ ]	As [ ]	λ_{max} [nm]
TBBPA	3.34	-	1.000	210
HBCD	5.75	6.394	1.000	210
BDE-209	10.90	9.104	1.000	225
deBDethane	12.74	2.440	0.875	225

t_R: retention time [min]; Rs: resolution factor [ ]; As: asymmetry factor [ ]; λ_{max}: maximum wavelength [nm]

For the analysis of the samples from the batch experiments with individual test compounds, isocratic elution (**Ch. 3.6.3**, **Tab 3.4**) was applied with different composition of the mobile phases in order to get the best compromise between a short total running time and the best separation of the respective test compound from the matrix peaks. Mixtures of methanol and water (75:25 v/v and 85:15 v/v) were used for TBBPA and HBCD, respectively. More polar mobile phases than in the multiple compounds methods generated a prolongation of the retention times of the test compounds and thus minimized the overlap with early-eluting matrix peaks (**Fig. 4.2**). In comparison to the gradient elution program, the t_R was prolonged from 3.34 to 10.35 min for TBBPA and from 5.75 to 12.64 min for HBCD. Furthermore, the mobile phase for TBBPA analysis was adjusted to pH 4 in order to reduce peak tailing by partial dissociation. Pure methanol and a mixture of methanol/isopropanol (IPA) (1:1 v/v) were applied for BDE-209 and deBDethane, respectively. IPA in the mobile phase enhanced the elution power and further improved the asymmetry factor of deBDethane from 0.875 to 1.000 together with an increase of the column temperature to 40 °C.

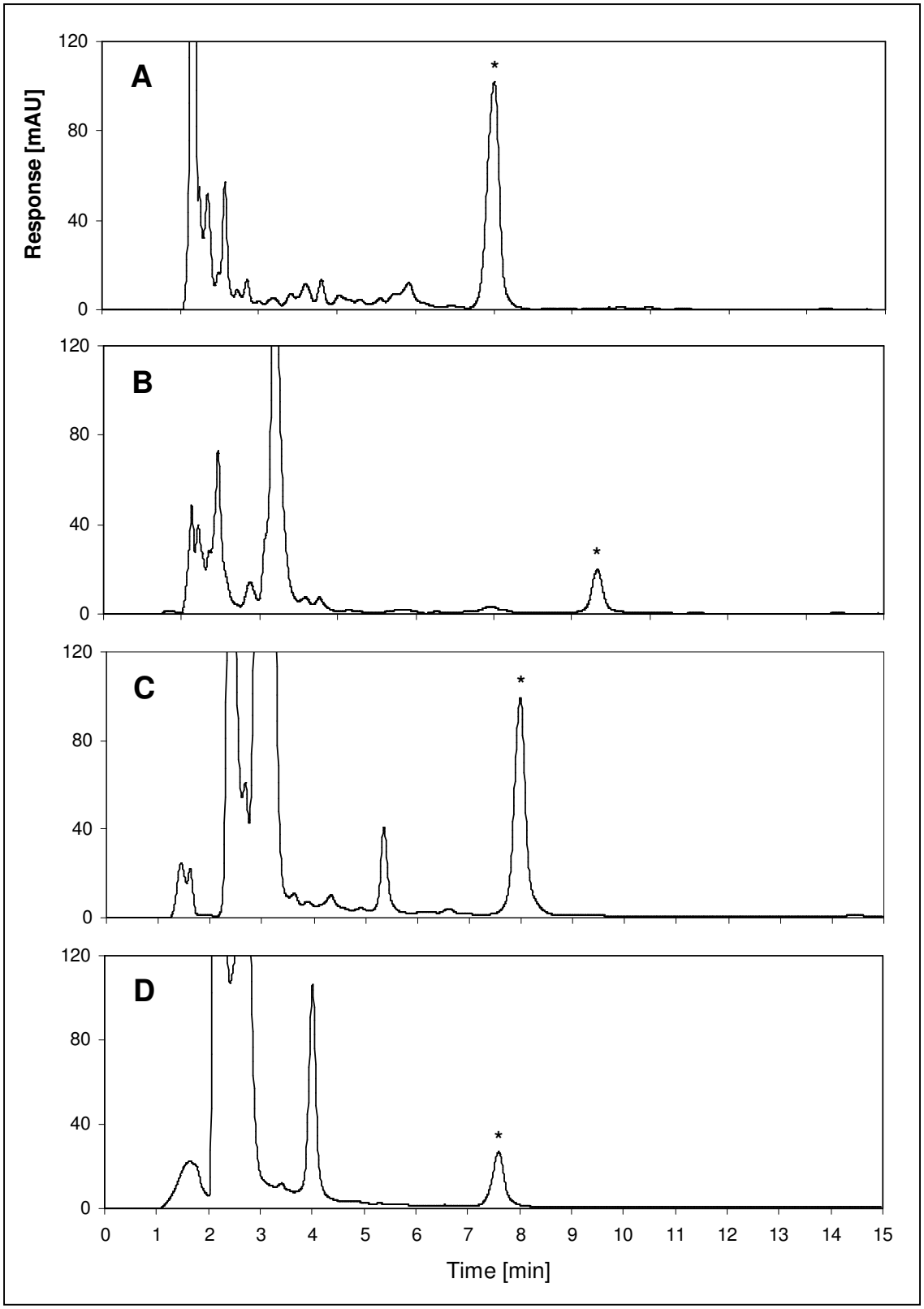


Figure 4.2: HPLC/DAD-chromatogram of test compounds (*) which separated from the matrix peaks under isocratic conditions. **A.** TBBPA (25 ng/µL), **B.** ∑HBCD (250 ng/µL), **C.** BDE-209 (25 ng/µL), and **D.** deBDethane (25 ng/µL)

4.1.2 Clean up

GPC

For the removal of high molecular organic matrix compounds that were co-extracted in large quantities with the lipophilic test compounds, such as humine substances, pigments, high molecular fats and oils, and high molecular hydrocarbons, GPC was tested. GPC separates molecules according to their size. Big molecules are less retained and eluted first because large size molecules cannot enter the pores of the stationary phase (gel). Small molecules are strongly retained because they enter several pores while passing the column. The fractionation profile of a BFR standard solution (**Fig. 4.3**) showed that the test compounds were eluted from eluates 5 to 12 (100 to 300 mL) and could be separated into two fractions. The first fraction was eluates 5 to 6 (100 to 150 mL), which contained TBBPA and HBCD, and the second fraction was eluates 6 to 12 (150 to 300 mL) which contained BDE-209 and deBDethane.

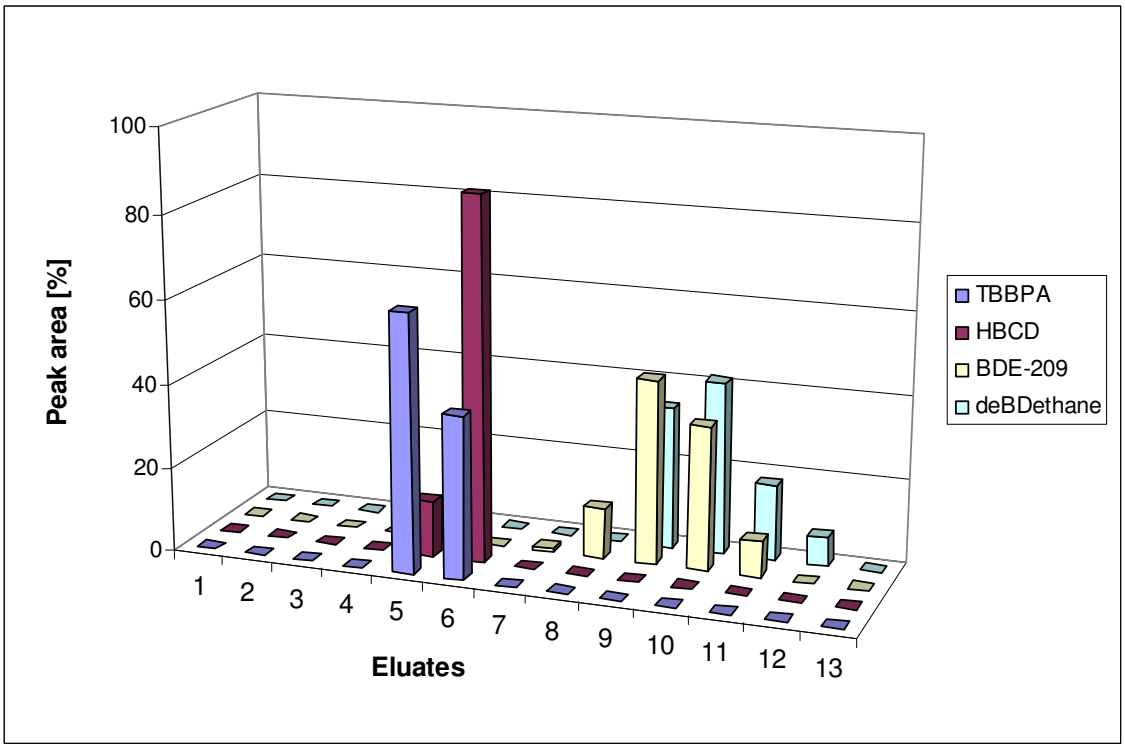


Figure 4.3: GPC elution profile of the test compounds: eluates 1-4 (100 mL) discarded, eluates 5-6 (100 to 150 mL) collected as fraction 1, eluates 7-12 (150 to 300 mL) collected as fraction 2. GPC column: Bio-Beads SX-8 (200-400 mesh); flow rate: 5 mL/min; each eluate corresponds to 5 min elution (= 25 mL)

However, different than expected, the test compounds were eluted in a sequence that corresponded to their polarity (TBBPA > HBCD > BDE-209 > deBDethane) and not to their molecular size (TBBPA < HBCD < BDE-209 < deBDethane). A similar behaviour was observed for PAHs during GPC cleanup with Bio-Beads SX-8 of sediment extracts. The PAHs were eluted after analytes with smaller molecular size like phenols, HCH, etc. (Kolb et al., 1995). These observations suggest chemical interactions between the stationary phase and the analytes in addition to the separation according to molecule size.

The fractionation of a blank sludge extract showed that according to theory that the large molecular sized organic matrix compounds, like lipids, hydrocarbons, and humic substances, were eluted before the first-eluted test compounds (TBBPA and HBCD). However, part of these co-extractants (mostly lipids) still overlapped with fraction 1 and produced a yellow-colour of the sample solution. Thus, GPC provided only a partial removal of co-extractants and did not sufficiently eliminate interference with TBBPA and HBCD. In contrast, a more efficient cleanup effect was obtained for fraction 2 containing BDE-209 and deBDethane, which was indicated by the clear colour of the sample solutions. Accordingly, the HPLC-DAD chromatograms of the GPC fraction 1 (**Fig. 4.4**) showed still a high baseline and matrix peaks that were interfering with the test compounds.

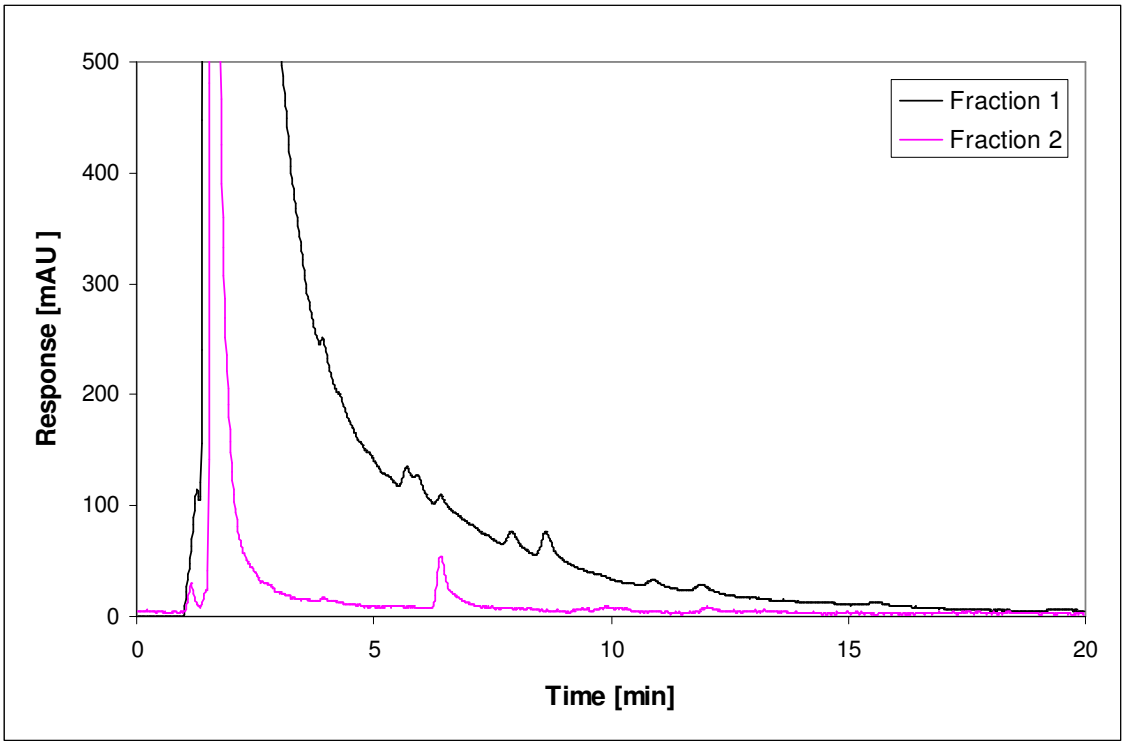


Figure 4.4: HPLC-DAD chromatogram of GPC fractions (1 and 2) of a blank sewage sludge sample (1 g d.s.)

In the fraction 2, the baseline was lower and only a few of matrix peaks were present in the chromatogram. Hence, the GPC cleanup for fraction 2 was sufficient for HPLC/DAD analyses of BDE-209 and deBDethane as those compounds eluted after 7 min in the chromatograms where the matrix interferences were low. Therefore, the fractionation of the GPC eluate minimized the number of steps in the cleanup procedure for these 2 test compounds. For fraction 1, additional cleanup was necessary in order to remove residual lipids from the eluate.

Column chromatography

In order to hydrolyze and oxidize rests of matrix compounds, a column chromatography with acidic impregnated silica gel was tested. A combination with basic-impregnated silica column, which is often described in literature for PCBs (e.g. Guo et al., 2009), was not chosen in order to avoid debromination of HBCD, which was reported by de Boer et al. (2001). Nevertheless, TBBPA was reported to be stable under this alkaline condition (de Boer et al., 2001). In the first step, it was tested if TBBPA and HBCD at a spiked concentration of 50 mg/kg d.s. (TBBPA) and 250 mg/kg d.s. (HBCD) were stable during cleanup with an acidified-silica column (44% H_2SO_4, w/w). A neutral silica column (5% H_2O, w/w) was run in parallel for comparison. The elution was performed with 150 mL of n-hexane/DCM (1:1, v/v). The test showed no obvious loss of TBBPA and HBCD in the acidified-silica column in comparison to the neutral-silica column (**Tab. 4.2**).

Table 4.2: Recovery rates in % of TBBPA and HBCD at column chromatography (n = 2)

Test compounds	Recovery ± RSD [%]	
	Neutral silica[1]	Acidified silica[2]
TBBPA	92 ± 0.22	91 ± 0.92
HBCD	99 ± 0.97	98 ± 0.83

[1]water deactivated (5% H_2O, w/w); [2]acid impregnated (44% H_2SO_4, w/w); elution with 150 mL n-hexane/DCM (1:1, v/v)

Due to the presence of the active hydroxyl groups in TBBPA and silica gel, a strong interaction of TBBPA with activated silica gel occurs. This interaction produces strong retention of TBBPA on the silica column. Therefore, it was necessary to modify the silica gel by deactivation with water. Different deactivation levels of silica were tested. A deactivation of 10% w/w produced a considerable retention of TBBPA. Only about 60% TBBPA was eluted in comparison to 98% of HBCD (**Fig. 4.5**). Furthermore, at a deactivation level of 20%,

recovery rates of 111% TBBPA and 110% HBCD were obtained. However, this high deactivation had the disadvantage that the polar matrix components were not sufficiently retained. Thus, less cleanup was obtained. Finally, a deactivation level of 15% was chosen as it produced acceptable recoveries of 91% TBBPA and 103% HBCD, and the coextractant were removed sufficiently. In contrast to TBBPA, the recovery rates of HBCD in three different deactivation levels of silica columns were always >98%.

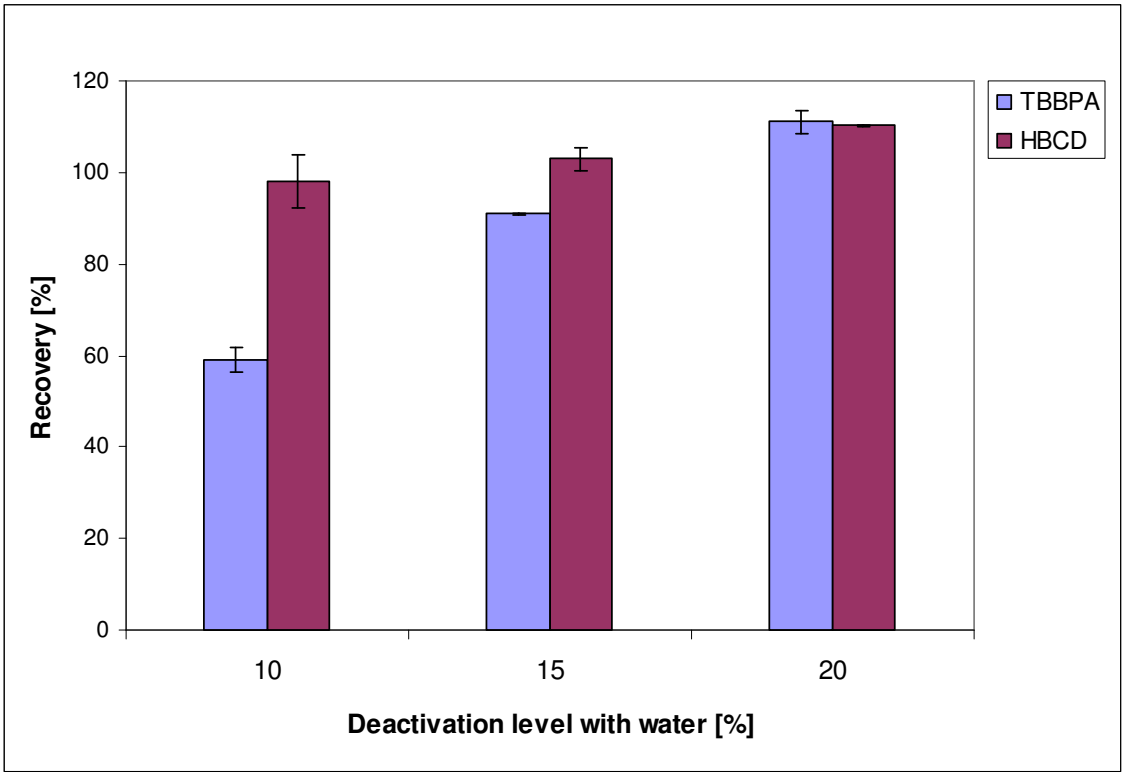


Figure 4.5: Recovery rates in % of TBBPA and HBCD in a silica cleanup column with different deactivation levels with water (n = 2), elution with 150 mL n-hexane/DCM (1:1, v/v)

Finally, a sufficient cleanup of GPC fraction 1 containing TBBPA and HBCD was achieved with the combined stationary phase of acidic and neutral silica gel (15% H_2O, w/w) using n-hexane/DCM (1:1 v/v) as mobile solvent. A combination of n-hexane with more polar DCM was applied as it has a stronger elution power for TBBPA. Replacement of DCM by other more eco-friendly solvents, e.g. ethyl acetate, was not possible due to incompatibility of these solvent with acid. The retention of oxidized coextractants on the silica column were recognizable by the dark colour of the upper part of the column (**Fig. 4.6A**) and the clear colour of the extract (**Fig. 4.6B**). A sufficient cleanup was further confirmed by the HPLC/DAD chromatograms after GPC and silica cleanup of the sludge extract. In

comparison with the samples, which were only cleaned up by GPC, the chromatograms of HPLC/DAD showed a lower baseline and only a few matrix peaks (**Fig. 4.7**).

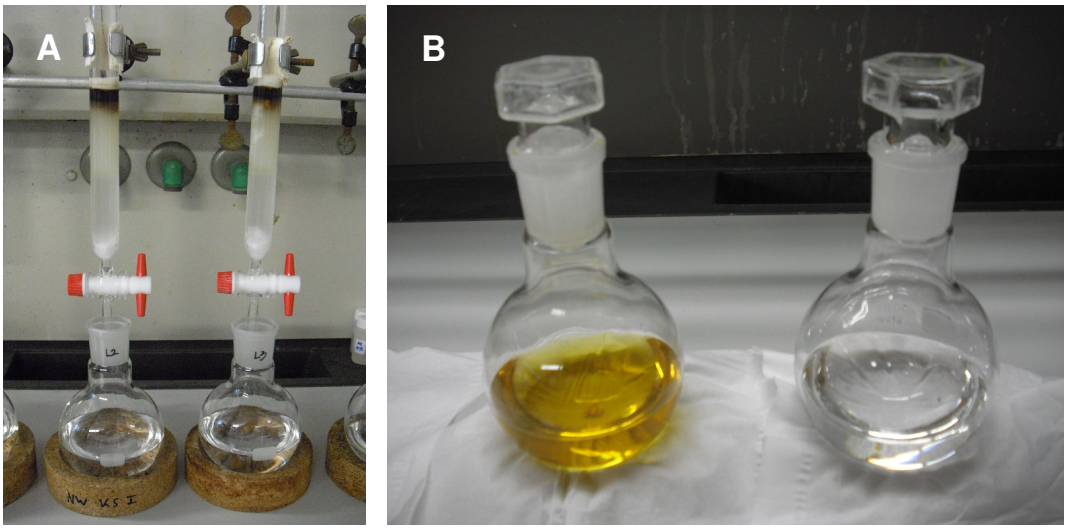


Figure 4.6: A. Multi-layer silica column cleanup of GPC fraction 1. Retained co-extractants are visible as a dark layer at the upper part of column. **B.** GPC fraction 1 before and after silica cleanup. **Left:** before cleanup (yellow solution); **Right:** after cleanup (clear solution)

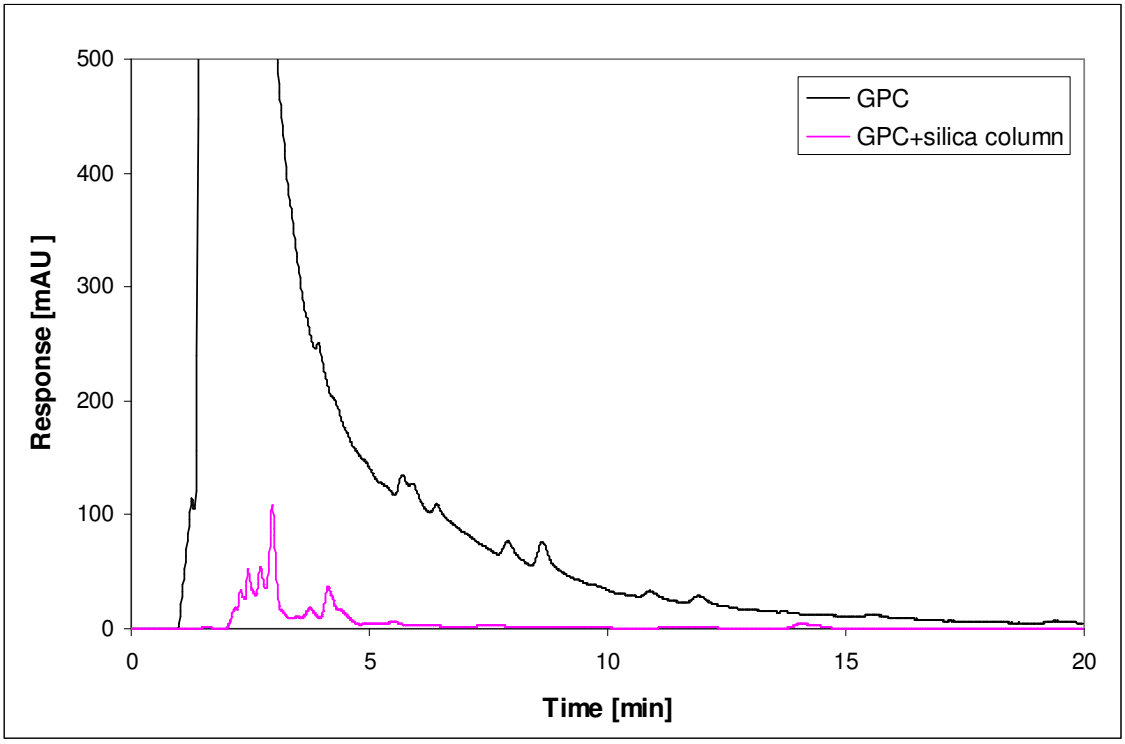


Figure 4.7: HPLC-DAD chromatogram of a blank sewage sludge sample (1 g d.s.) after GPC (fraction 1) and after silica column cleanup

4.1.3 Extraction

Prior to extraction, sample pre-treatment subjected for water removal from the matrices was carried out. For this purpose, freeze-drying was used. Different freeze-drying times were compared in order to find out the optimum drying time (**Fig 4.8**). After 8 h drying time, the dry mass was 73%, which was still too high for extraction. Meanwhile, after 12 h sludge with 95% d.s. was obtained. The extension of the drying time to 24 h even removed another portion of water (up to 99%). However, this was less time-efficient. Furthermore, a longer drying time increases the risk to loose test compounds from the matrix. Therefore, a drying time of 12 h was chosen as the best compromise.

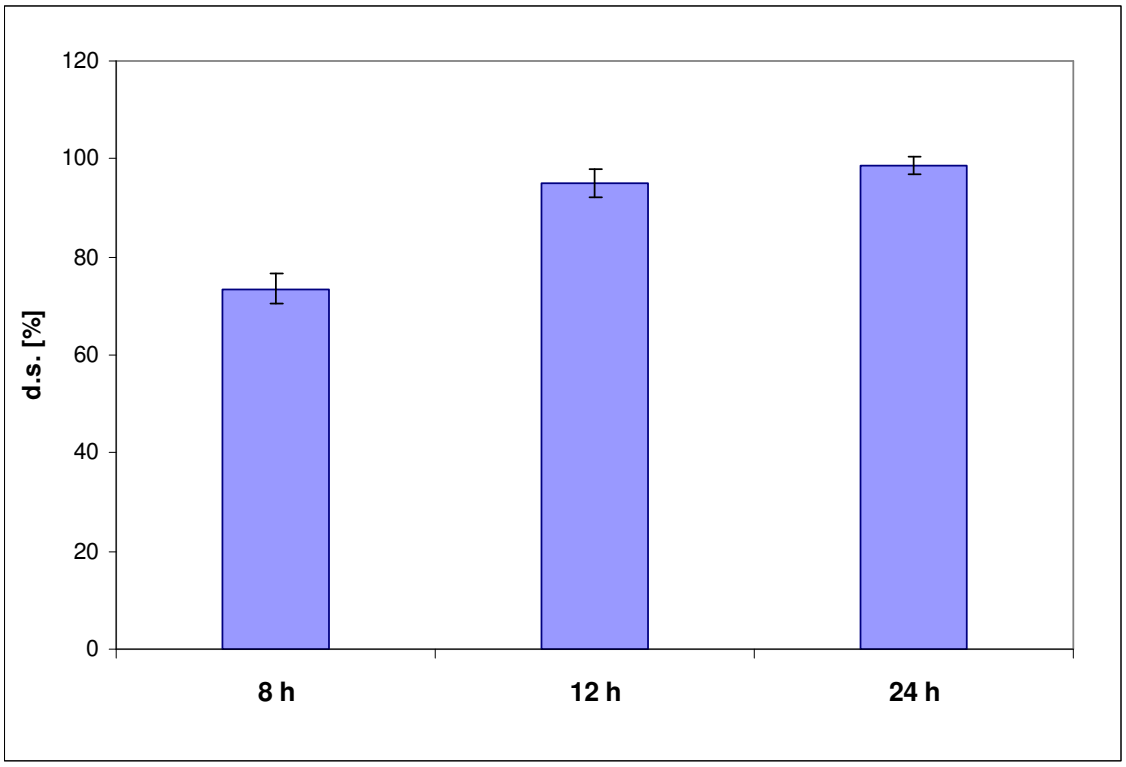


Figure 4.8: Water content of freeze-dried sewage sludge (in % d.s.) obtained from different drying time (n = 4)

During freeze drying, the frozen water was gently removed by sublimation process at low pressure (0.05 mbar). However, the analytes with higher vapor pressures were retained. Advantages of this method include an efficient removal of water without the need to add chemical drying agents. Furthermore, no heat generation prevents volatilization of the analytes considering semi-volatile properties of some BFRs, especially HBCD. By the freeze drying process, the water content of the sludge matrices was reduced from around 97% to

<10%. A small part of 2 to 8% of water still remained, which was presumably intracellular water, as intracellular water is known to represent around 8% of total sludge moisture (Chen et al., 2002). In order to adsorb the rest of water content which was not removed, anhydrous Na_2SO_4 was homogenously mixed with the freeze-dried sludge by grinding.

Soxhlet extraction was chosen for the extraction procedure due to its effectiveness for the extraction of BFRs from sewage sludge as reported by many authors (Covaci et al., 2003; Eljarrat and Barceló, 2004; Díaz-Cruz et al., 2009). For TBBPA, the pH during extraction is an important factor considering that TBBPA has two pKa values, 7.5 and 8.5 (Covaci et al., 2008). Under neutral environments, a substantial part of the TBBPA is deprotonated and present in its dissociated state, which produces losses during extraction, when a polar solvent is used or trace of water is present. For this reason, the pH of TBBPA extraction was set ≤5 by adding 200 µL of sulfuric acid to the sludge after grinding with anhydrous Na_2SO_4. Under this condition, most of TBBPA will remain in the form of non-dissociated species and facilitate an efficient extraction towards organic solvents. The extraction efficiency of the 4 test compounds using different solvents at Soxhlet extraction is shown in **Fig. 4.9**.

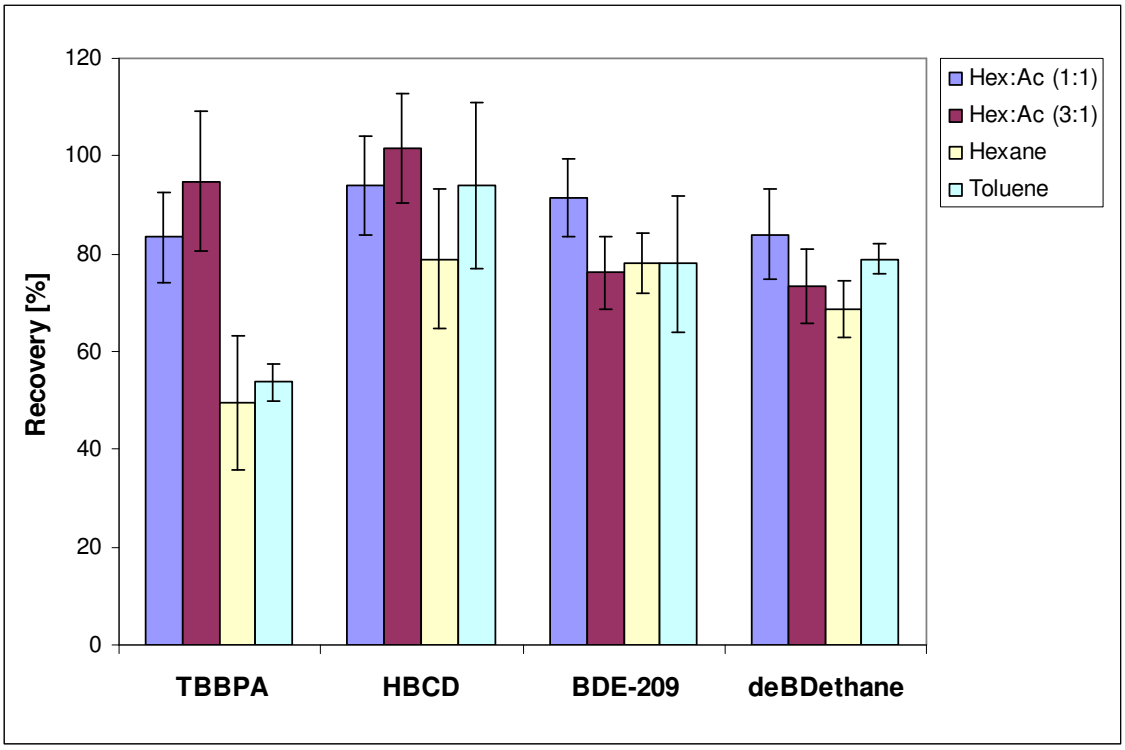


Figure 4.9: Recovery rates in % of the test compounds from raw sludge matrix with different solvents (n = 4)

Lower recoveries by using a single solvent of n-hexane and toluene were obtained, especially for TBBPA and HBCD. This might be a consequence of a low accessibility of these non-polar solvents to the inner part of the sewage sludge, which contains many polar groups, such as amines, phenols, and carboxylic acids. In case of toluene, high amount of co-extractant was observed by very dark extracts. Furthermore, this solvent was more complicated to handle, i.e. more difficult to evaporate due to its relative high evaporation temperature (111 °C). Finally, the mixture of n-hexane/acetone (1:1, v/v) was chosen as this mixture produced a lower coefficient of variation as the 3:1 (v/v) mixture. Better recovery for BDE-209 and deBDethane were obtained with 1:1 (v/v) mixture of n-hexane/acetone. Furthermore, the 1:1 (v/v) mixture of n-hexane/acetone is an azeotropic solvent (Stavroudis, 2006), which means that it maintains a constant composition with a low boiling point of 49.8 °C. Whereas, the 3:1 (v/v) mixture of n-hexane/acetone changes its composition during evaporation until it reaches the azeotropic composition of 1:1 and thus changes its boiling point to 49.8 °C. This result was in agreement with the work of Morris et al. (2006). They reported that mixtures of n-hexane and acetone (1:1 or 1:3, v/v) produced high recoveries for TBBPA and HBCD.

4.1.4 Validation of the analytical method with HPLC/DAD

Accuracy and precision

Accuracy and precision for the whole method, including extraction, cleanup, and HPLC/DAD analysis were determined by fortification experiments. For that purpose, sludge samples were fortified at two concentration levels (5 and 50 mg/kg d.s.). These fortification levels were chosen considering the analytical range of HPLC/DAD and also the spiking level for the batch tests (50 mg/kg d.s.). The results of the test are reported in **Tab. 4.3**.

Table 4.3: Recovery rates of BFR test compounds from the spiked sewage sludge samples (n = 4)

Test compounds	Recovery ± RSD [%]	
	5 mg/kg d.s.*	50 mg/kg d.s.**
TBBPA	80 ± 4	76 ± 2
HBCD	83 ± 3	89 ± 6
BDE-209	68 ± 3	89 ± 1
deBDethane	80 ± 3	86 ± 2

*HBCD: 25 mg/kg d.s.; **HBCD: 250 mg/kg d.s.

For a fortification level of 5 mg/kg d.s., the recovery rates ranged from 68 to 83%, and for a level of 50 mg/kg d.s. from 76% to 89%. In case of TBBPA with recovery rates of 76 and 80%, losses occurred during silica cleanup, where recovery was around 90%. Thus, other 10-16% losses were caused by extraction. According to the criteria of European Union (SANCO/2007/3131), the results were in the acceptable range of 70-120% with RSD ≤20%, with the exception of the little lower recovery rate (68%) of BDE-209 at the lower fortification level of 5 mg/kg d.s.

A further fortification experiment was performed under the condition of storage at -32 °C for 32 d in order to evaluate if the recovery rates were significantly reduced by storing the samples in a freezer until preparation. This test was carried out at a spiking level of 50 mg/kg d.s. (250 mg/kg d.s. for HBCD). The results of this test are reported in **Fig. 4.10**.

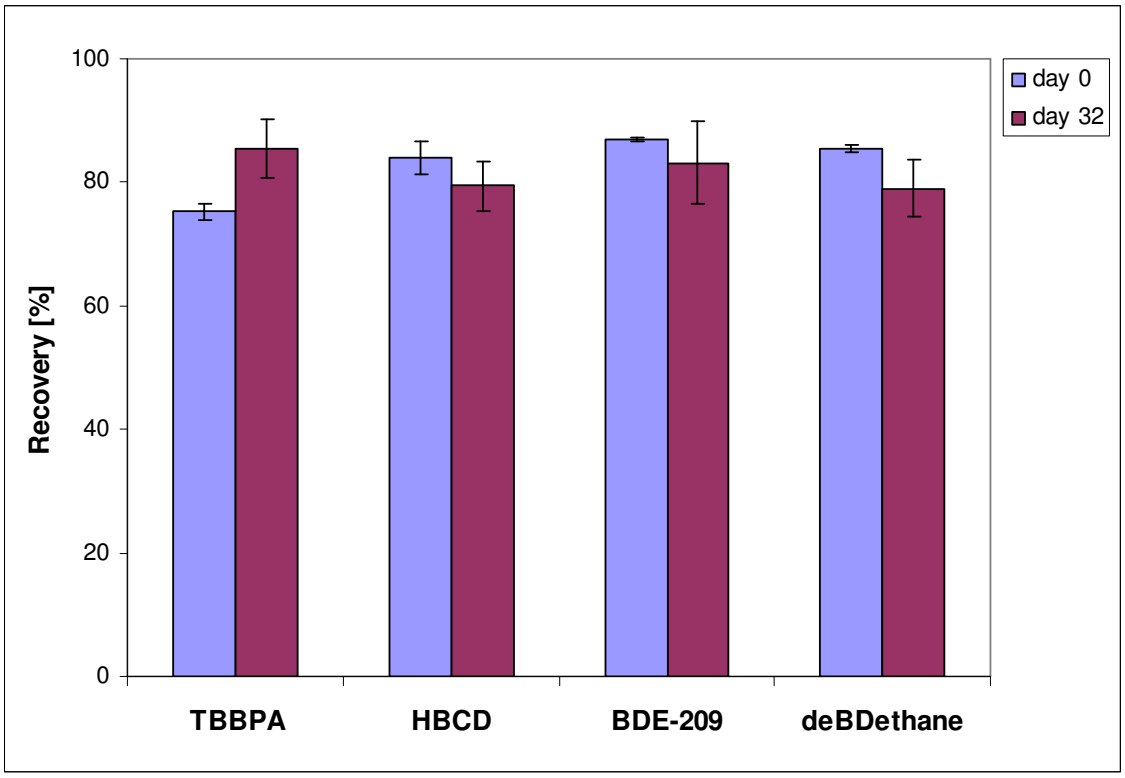


Figure 4.10: Recovery rates of spiked test compounds from fresh samples (direct extraction, day 0) and aged samples (extraction after 32 d), (n = 2)

Only a small reduction of 4 to 6% of the extraction efficiency was observed for the 3 test compounds (HBCD, BDE-209, deBDethane) after storage of the samples. An increase of the recovery rate of 10% was obtained for TBBPA. In general, the extraction of stored samples produced higher RSD values, ranging from 4.10 to 6.54% than fresh samples, where RSD

value ranged from 1.26 to 5.03%. However, in total the results show that the storage of the samples in the freezer until sample preparation did not change the recovery rates significantly.

Limits of detection and quantification

For the calibration of the test compounds, six different concentrations were measured with HPLC/DAD. Good linearity within the concentration range was obtained for all analytes with determination coefficients (R^2) >0.997 (**Tab. 4.4**).

Table 4.4: Calibration curves and linearity for the HPLC/DAD method

Test compounds	Conc. range [ng/µL]	Regresion equation	R^2
TBBPA	1 -100	y = 72.9807x + 35.8816	0.99992
ΣHBCD	10 -1000	y = 1.3603x + 2.0937	0.99942
BDE-209	1 -100	y = 42.7218x + 72.0587	0.99928
deBDethane	2 - 50	y = 17.9216x + 23.0462	0.99725

The limits of detection (LOD) and quantification (LOQ) were calculated as the minimum amount of test analyte that produced a peak with a signal-to-noise response of 3 and 10, respectively (**Tab. 4.5**).

Table 4.5: Limits of detection (LOD) and quantification (LOQ) of the HPLC/DAD method for reference standard solutions

Test compounds	LOD [mg/kg]	LOQ [mg/kg]
TBBPA	0.91	3.03
ΣHBCD	23.7	79.1
BDE-209	2.66	8.86
deBDethane	3.56	11.9

LOD: limit of detection; LOQ: limit of quantification

The sensitivity of HPLC/DAD detection was ranging from a LOD of 0.91 mg/kg for TBBPA and 23.7 mg/kg for HBCD. These values were used as the basis to determine the spiking levels for the batch experiments.

4.1.5 GC/MS

For the batch tests, the quantification of the test compounds was performed by using HPLC/DAD. In addition, GC/MS, which was operated in SIM mode, was used for further confirmation of the identity and for the quantification of the test compounds. Furthermore, for the identification of the possible degradation products GC/MS was operated in a full-scan mode. The recorded mass spectra were compared with spectra of the instrument's spectra library (NIST).

TBBPA. TBBPA derivatisation was carried-out by acetylation by using acetic anhydride in order to produce less polar acetyl-TBBPA (ac-TBBPA). The formation of the derivatized TBBPA was confirmed by the detection of the adduct ion $[M+C_2H_3O]^+$ with *m/z* 585.9, in comparison to spectra of native TBBPA where this peak was not detected (**Fig. 4.11**). The yield of derivatisation was 97%. Thus, the chromatogram of a derivatized TBBPA showed still a small peak of underivatized TBBPA (peak 1; t_R = 13.23) aside the main peak of ac-TBBPA (peak 2; t_R = 13.47). Derivatisation increased the response of the TBBPA by the factor of 5 on the basis of peak area and prevented peak tailing.

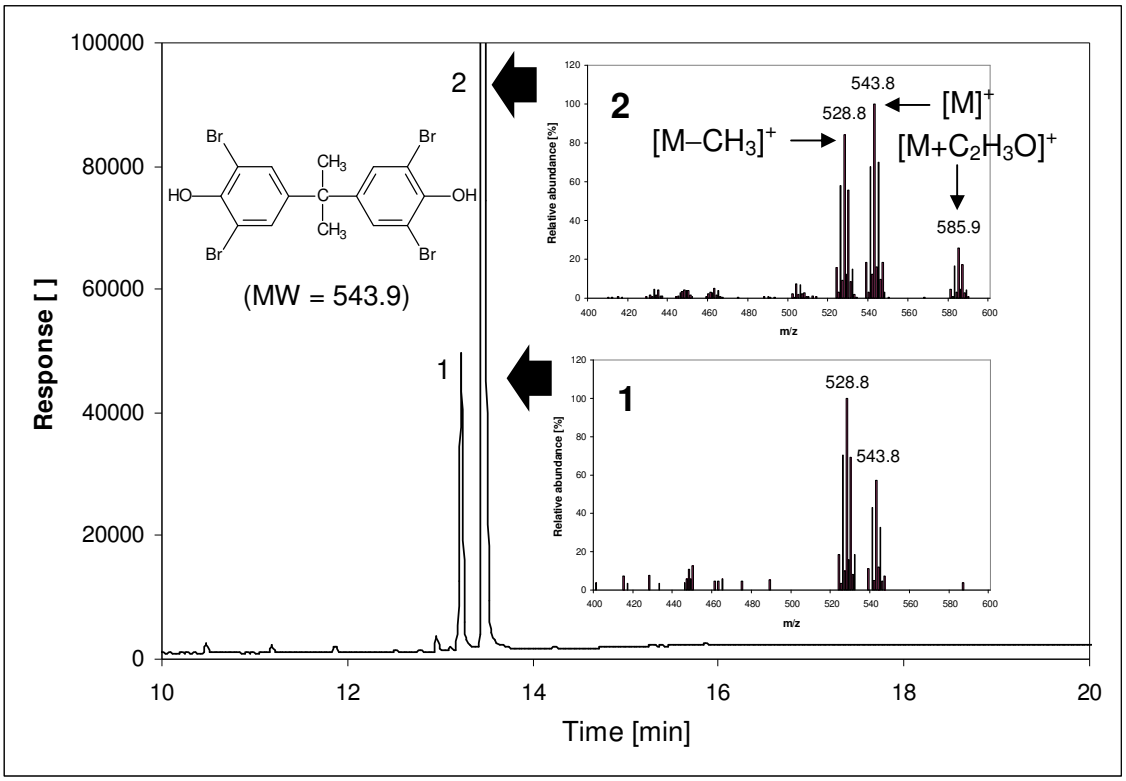


Figure 4.11: GC/MS (EI, full-scan mode) chromatograms and corresponding spectra of TBBPA standard: **1.** Underivatized TBBPA (peak 1), **2.** Derivatized TBBPA (peak 2)

The EI-MS spectrum of TBBPA showed a molecular ion $[M]^+$ with *m/z* 543.8 and followed by a less intense ion fragment with *m/z* 528.8. This fragment ion corresponds to the loss of a methyl group $[M\text{-}CH_3]^+$. This molecular ion $[M]^+$ represents the base peak of the mass spectrum with the characteristic isotopic distribution pattern of four bromine atoms.

HBCD. The EI-MS chromatogram of HBCD (**Fig. 4.12**) showed three main peaks with t_R of 12.19, 13.57, and 15.59.

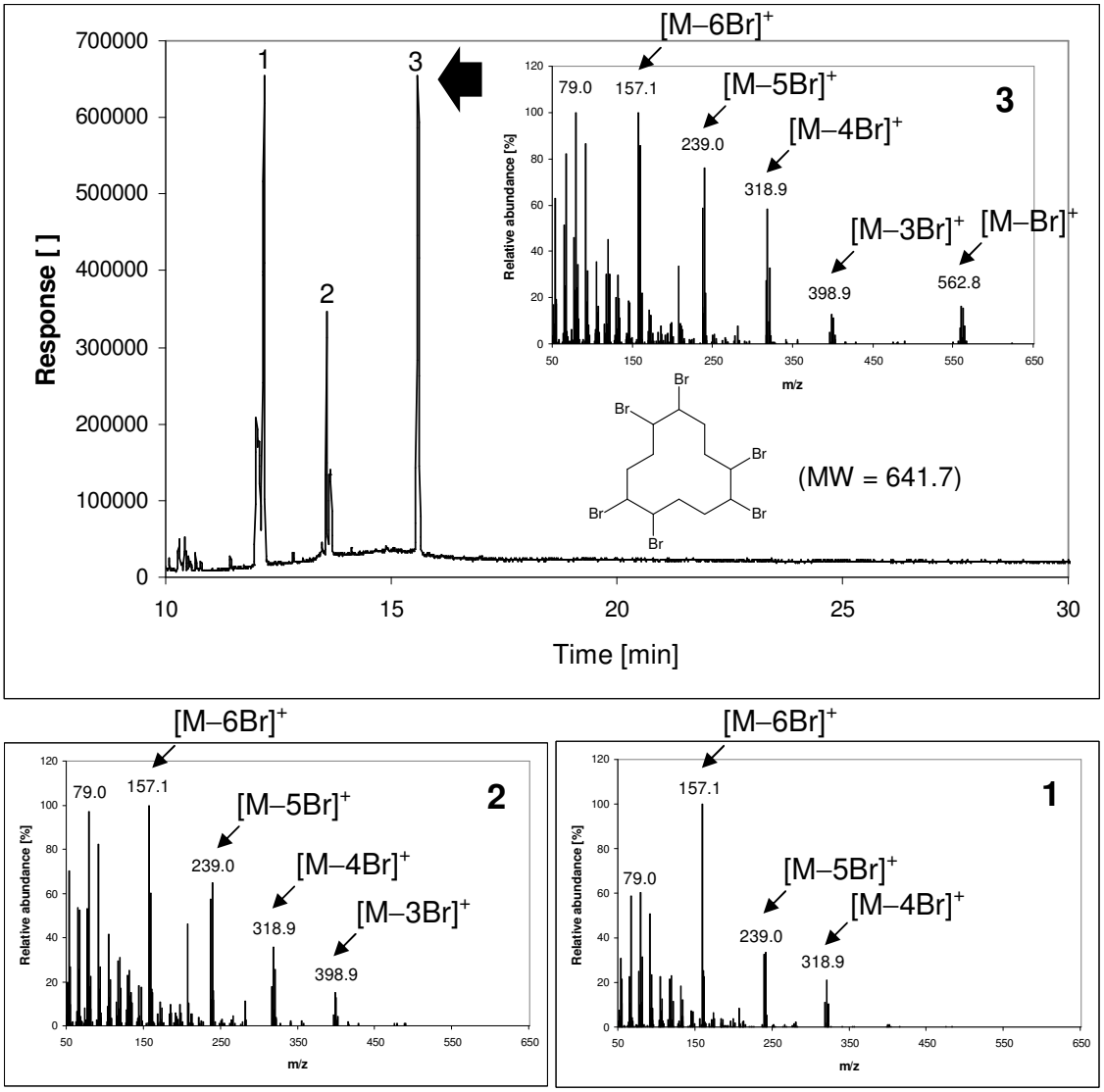


Figure 4.12: GC/MS (EI, full-scan mode): chromatogram of HBCD standard (α, β, γ-HBCD) and spectra of the detected peaks (1, 2, 3)

In the spectrum of peak 3 (t_R = 15.59) the fragment of *m/z* 562.8 was the signal with the highest mass and had an isotopic cluster of five bromine atoms $[M–Br]^+$. Thus, no signal of a

molecule ion of HBCD with *m/z* 641 was detected. Further signals of *m/z* 398.9, *m/z* 318.9, *m/z* 239.0, and *m/z* 157.1 showed bromine clusters with mass differences of 80 Da, respectively, indicating consecutive losses of 3, 4, 5, and 6 bromine atoms. For peak 2 (t_R = 13.57) ion fragments of *m/z* 398.9, *m/z* 318.9, *m/z* 239.0, and *m/z* 157.1 with bromine cluster were observed. The signal with the highest mass (*m/z* 398.9) indicated the loss of 3 bromine atoms, while *m/z* 318.9, *m/z* 239.0, and *m/z* 157.1 indicated a consecutive losses of 4, 5, and 6 bromine atoms. In case of peak 1 (t_R = 12.19), fragment ions of *m/z* 318.9, *m/z* 239.0, and *m/z* 157.1 were observed, indicating consecutive losses of 4, 5, and 6 bromine atoms. From the spectra, it is assumed that peak 1 and 2 were the thermal degradation products of HBCD, which were formed during the chromatographic process. In all spectra of HBCD, an intensive bromine peak at *m/z* 79.0 was monitored. This signal was used for quantification in SIM mode.

As it was not possible to prevent the partial thermal degradation of HBCD in GC/MS analysis, the reproducibility of the degradation was tested in order to allow a reproducible quantification. For that purpose, consecutive injections of an HBCD standard (20 ng/µL) were performed (**Fig 4.13**).

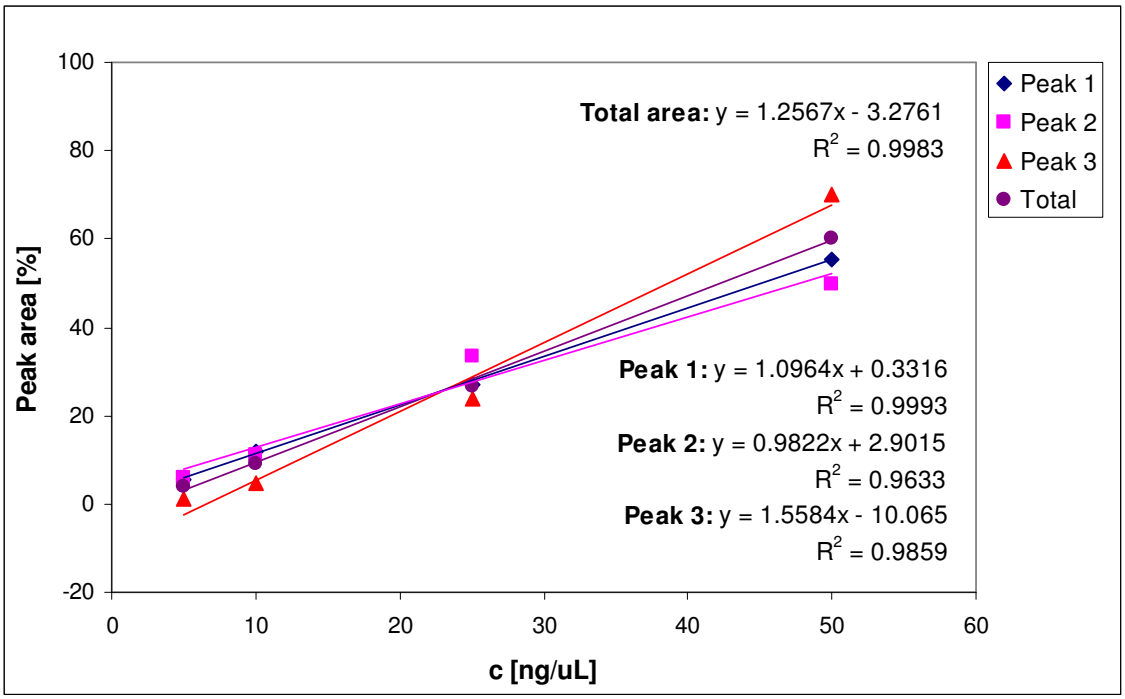


Figure 4.13: Linear correlation of peak areas versus concentration (5-50 ng/µL) of the three main peaks of the HBCD standard in GC/MS analysis

For the peak areas of peak 1, 2, and 3, ratios of 10.2, 20.8, and 69.0% to the total peak area were obtained, respectively. The RSD of these ratios were 9.7, 14.4, and 8.9%, respectively. However, calibration based on the total peak area was the best option, as it gave the best linearity of the calibration curve with R^2 = 0.9983 in a concentration range of 5 to 50 ng/µL and the lowest RSD of 8.2% (n = 4).

BDE-209. The GC/EI-MS spectrum of BDE-209 is shown in **Fig. 4.14**. The major ions formed were $[M]^+$ and $[M–2Br]^+$ which were used for their identification and quantification.

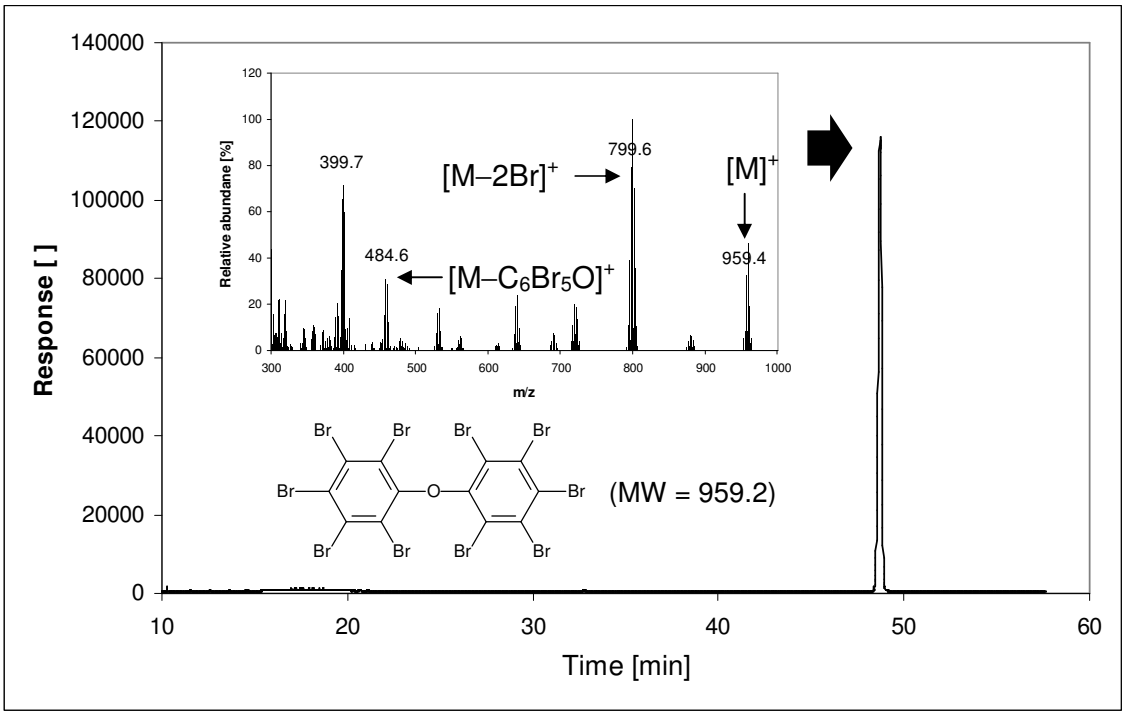


Figure 4.14: GC/MS (EI, full-scan mode) chromatograms and spectra of BDE-209 standard

The ion cluster at *m/z* 799.6 was the base peak. It was formed by the loss of two bromine atoms to a $[C_{12}H_1Br_8O]^+$ fragment. The relatively high abundance of $[C_{12}H_1Br_8O]^+$ fragment is explained by the formation of the more stable dibenzofuran ion (Teclechiel, 2008). The molecular ion cluster $[M]^+$ at *m/z* 959.4 was less intense than the fragment at *m/z* 799.6. A further ion fragments of *m/z* 484.7 with higher intensity was the $[C_6Br_5O]^+$ fragment, which was formed by a cleavage of the ether bond and loss of five bromine atoms $[M–C_6Br_5O]^+$.

DeBDethane. At GC/MS analysis of deBDethane, with conditions that were applied for BDE-209 (final GC temperature of 300 °C), neither the test compound nor degradation product were detected even if the elution time was extended to 60 min. So, it was assumed that the

temperature of 300 □C was not high enough for evaporation of the compound. Thus, a DB5-HT (30 m × 0.25 mm i.d; 0.1 µm film thickness) column which can tolerate GC oven-temperature up to 400 °C was tested. By using a thin film stationary phase column of DB5-HT with a temperature program up to 325 °C, and an injection volume of 2 µL, deBdethane could be finally detected. However, deBDethane showed a peak response that was a factor of 2.8 lower than the response of BDE-209 in full-scan mode (**Fig. 4.15**). An obvious lower response of deBDethane compared to BDE-209 was also reported by Konstantinov et al., (2006) and Kierkegaard et al. (2009) in LRGC/EI-MS full-scan when the molecular ions were monitored.

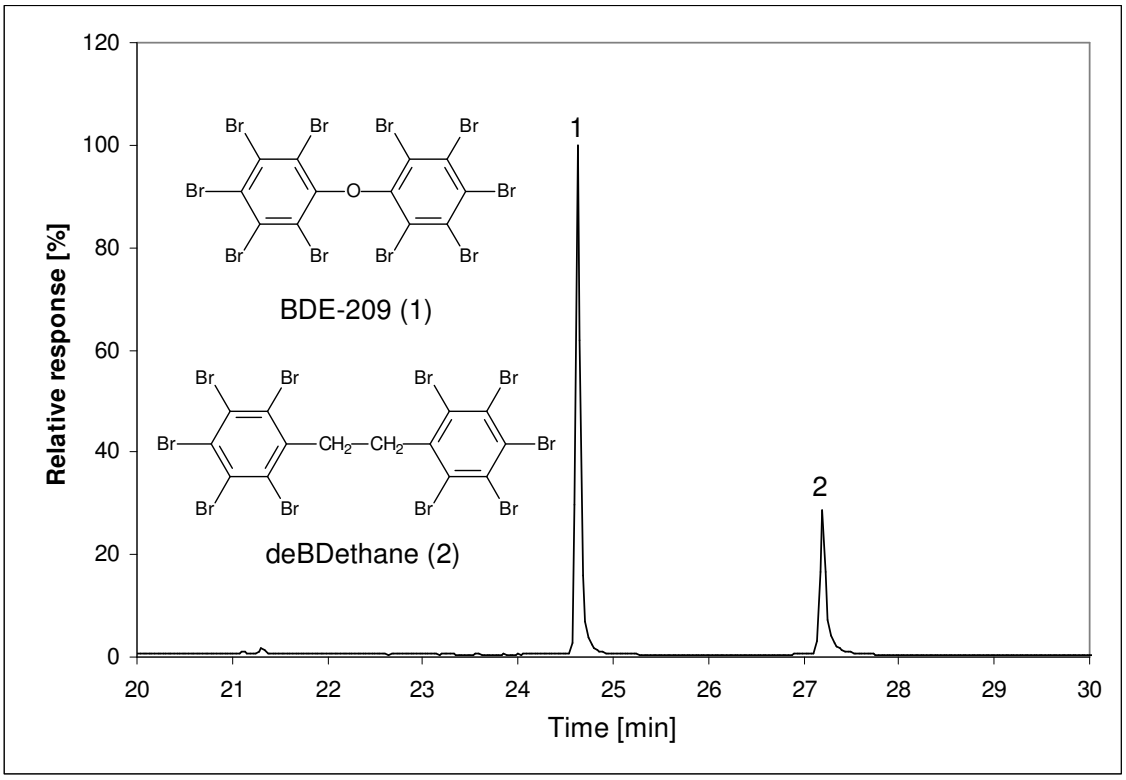


Figure 4.15: GC/MS (EI full-scan mode) chromatogram showing a comparison of response of BDE-209 (peak 1) and deBDethane (peak 2). Column: DB5-HT (30 m × 0.25 mm i.d; 0.1. µm film thichness); temperature program: 110 °C (2 min) to 325 °C at 12.5 °C/min (22.5 min); standard: 10 ng/µL in n-hexane; injection volume: 2 µL

In the EI spectrum of deBDethane, the $[C_7H_2Br_5]^+$ fragment with *m*/*z* 484.6 was the base-peak with an isotopic pattern indicating five bromine atoms (**Fig. 4.16**). This fragment was formed by the cleavage of the ethyl bond and the loss of five bromine atoms yielding the pentabromobenzyl fragment. Less intensive fragments with *m*/*z* 405.7 (30%) and 326.8 (47%) were also identified, with a further loss of one or two bromine atoms forming

$[C_6Br_4CH_2]^+$ and $[C_6Br_3CH_2]^+$ fragments, respectively. The molecular ion $[M]^+$ with *m/z* 971.5 had only a relatively low-abundant of 5%. Further low abundant fragments at *m/z* 893.5, 813.5, 732.6, 651.6, 572.7 of the molecular ion were formed by the loss of one to five bromine atoms, respectively.

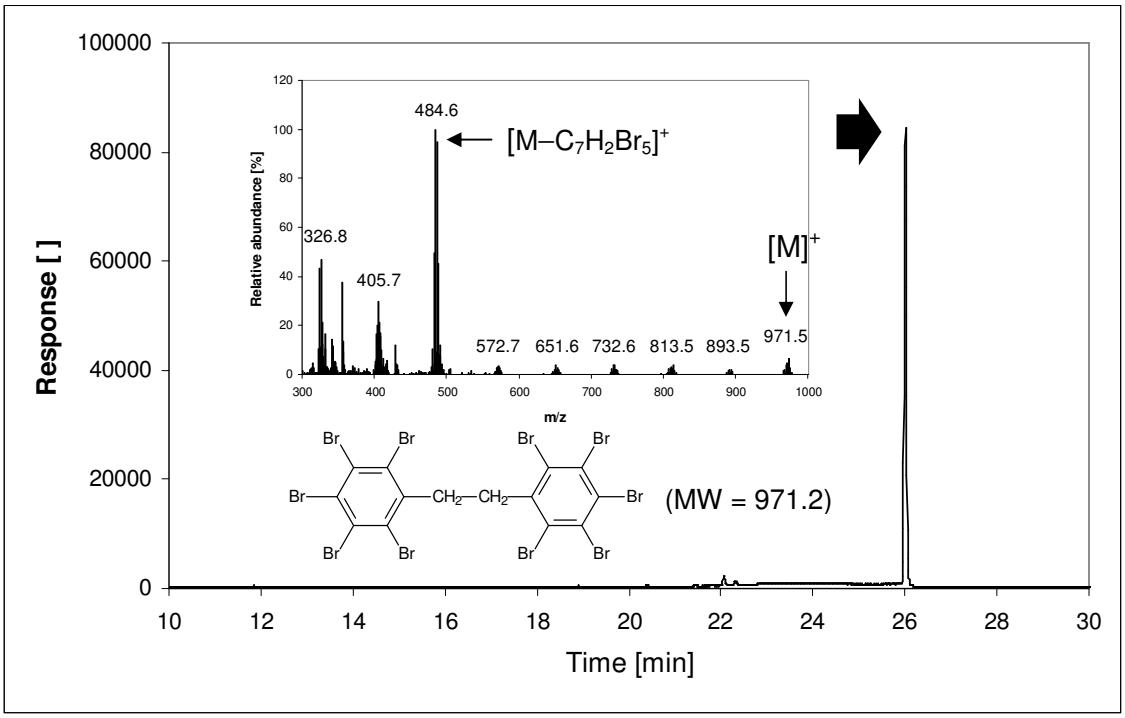


Figure 4.16: GC/MS (EI, full-scan mode) chromatograms and spectra of deBDethane standard

Peak parameters, as t_R and characteristic ion fragments, that were used for identification of test compounds are summarized in **Tab. 4.6**.

Table 4.6: Peak parameters of the test compounds at GC/MS analysis

Test compounds	t_R [min]	MW [g/mol]	Mass fragment[a]
ac-TBBPA	15.52	543.9	**544**, 529
HBCD	15.59[b]	641.7	319, 239, **157**
BDE-209	48.67	959.2	800, **400**
deBDethane	28.05	971.2	**485**

t_R = retention time [min]; MW = molecular weight [g/mol]; [a]The based peak are written in bold; [b]t_R of the third peak

4.2 Batch experiments

In order to study fate and behaviour of the selected test BFRs, studies with lab-scale batch systems were carried out which should simulate the real environmental conditions of sludge treatment in WWTPs. For this purpose, sludge was collected from WWTP of Braunschweig, characterized, spiked with the selected BFRs in concentrations that were significantly higher than the background levels, and then detected by HPLC/DAD. However, the chosen spike level was still in the high range of reported residues in sewage sludge.

4.2.1 Characterization of the sludge matrices

Raw sludge and digested sludge from WWTP of Braunschweig, which receives effluents from households and industries, were used for the study. The sludges were taken in three different sampling times for different batch tests. Prior the batch tests, the used sludges were characterized by their physical and chemical properties as summarized in **Tab. 4.7**.

Table 4.7: Physical and chemical properties of sludges from WWTP of Braunschweig

Parameters*	Raw sludge			Digested sludge	
	A	**B**	**C**	**B**	**C**
pH	5.5 ± 0.1	5.3 ± 0.1	5.6 ± 0.1	8.0 ± 0.1	7.3 ± 0.1
E_h	-20	5	20	-40	-80
d.s. [%][a]	3.95 ± 0.11	4.09 ± 0.11	4.91 ± 0.12	2.14 ± 0.03	2.58 ± 0.06
TOC [g/kg][a]	11.60 ± 0.29	11.00 ± 0.35	20.99 ± 0.02	8.61 ± 0.38	9.55 ± 0.47
TKN [g/kg][a]	2.54 ± 0.03	2.37 ± 0.03	3.55 ± 0.01	2.29 ± 0.01	2.96 ± 0.02
Ammonia [g/kg][a]	0.78 ± 0.04	0.71 ± 0.01	1.03 ± 0.01	1.46 ± 0.02	1.67 ± 0.01
C/N ratio	5.47	4.64	5.91	3.76	3.23
NO_3^- [g/kg][b]	0.16	-	0.06	0.29	0.09
NO_2^- [g/kg][b]	<LOD	-	<LOD	<LOD	<LOD
PO_4^{3-} [g/kg][b]	3.29	-	4.73	2.02	2.99
Cl^- [g/kg][b]	0.48	-	0.53	0.71	0.60
SO_4^{2-} [g/kg][b]	0.12	-	0.20	0.18	0.01

[a]Sludge collection: **A** = 07.08.2012, for AE test (raw sludge only); **B** = 14.11.2012, for AN test (raw+digested sludge); **C** = 28.02.2013, for AN-AE and LI test (raw+digested sludge); *Values are expressed on a fresh weight (f.w.) basis ([a]n = 2; [b]n = 1)

Physically, raw sludge was very heterogeneous, fibrous, and had a strong putrid odour, whereas digested sludge was more homogenous, darker in colour, and smelled like soil. Furthermore, **Tab. 4.7** shows an obvious difference between raw and digested sludge. Raw sludge was slightly acidic, with an average pH of 5.47, whereas digested sludge was neutral to basic with an average pH of 7.65. In raw sludge, due to its low redox potential of -20 mV, the hydrolises of lipids to fatty acid by facultative anaerobic bacteria already started. This process caused the acidic pH of raw sludge. Different sampling times did not give a significant difference of the pH values. The pH differed only by 0.3 for raw sludge and 0.7 for digested sludge. The redox potentials (E_h) of raw sludge were ranging from 20 to -20 mV and for digested sludge from -40 to -80 mV. These E_h values, which are < +200 mV, indicated that all sludge samples were originally under anaerobic conditions. In contrast to the pH values, the determination of E_h resulted in a higher variability of the results, which were probably caused by the difference of sampling conditions.

The dry substance (d.s.) value of digested sludge with 2.4% in average was obviously lower than raw sludge with 4.3% in average. In parallel, the content of organics in the sludge, measured as TOC, decreased during digestion as a consequence of carbon transformation to CH_4 and CO_2, which were released into the gas phase. Thus, digested sludge had a lower TOC of 8.6 to 9.6 g/kg f.w. compared to the TOC of 11 to 21 g/kg f.w. in case of raw sludge. The total N concentration (measured as TKN) was only slightly lower in digested sludge (in average 2.6 g/kg f.w.) than raw sludge (in average 2.8 g/kg f.w.). This indicated that N removal from raw sludge was not significant. However, digested sludge had higher ammonia content (in average 1.6 g/kg f.w.) than raw sludge (in average 0.8 g/kg f.w.) which indicated a formation of ammonia from proteins during the digestion process. In consequence of the high reduction of TOC during digestion and only small changes of total N, the C/N ratio of raw sludge was higher (5.3) than that of digested sludge (3.5).

Concentration levels of nitrite, nitrate, and inorganic salts (PO_4^{3-}, Cl^-, and SO_4^{2-}) in both sludges showed different trends. In case of nitrate, the concentrations at the different sampling times varied relatively high from 0.06 to 0.10 g/kg f.w. for raw sludge and 0.09 to 0.29 g/kg f.w. for the digested one, showing no obvious difference between raw and digested sludge. In general, an accumulation of nitrate in digested sludge is not expected, because of denitrification of nitrate to N_2 and the reduction to ammonia in a carbon-rich environment (Akunna et al., 1993). Nitrite concentrations were below the detection limit (<LOD) in both sludges. Lower concentrations of soluble PO_4^{3-} were observed in the digested sludge (2.5 g/kg f.w.) compared to the raw sludge (4.01 g/kg f.w.). One reason might be the precipitation of phosphate as magnesium ammonium phosphate (MAP) by the pH increase during

digestion. The concentrations of Cl^- and SO_4^{2-} were nearly constant in raw and digested sludge, which were 0.5 g/kg and 0.7 g/kg, respectively, for Cl^- and 0.16 g/kg and 0.10 g/kg, respectively, for SO_4^{2-}.

4.2.2 Process parameters of the anaerobic and aerobic batch tests

The batch systems were evaluated by regular monitoring of temperature, pH, and redox potential. Other parameters, such as d.s., TOC, and N, were also controlled. In case of anaerobic conditions, also the production biogas was monitored every day. During the anaerobic and aerobic batch test, stable performance in the batch reactors was observed from the first day of incubation of the sludge from WWTP, as inoculum, indicating that sludge was already well adapted.

Temperature

For the anaerobic batch test, thermophilic conditions (54 °C) were chosen as the digester of WWTP of Braunschweig was run at that temperature. Thus, the microbial community of the digested sludge used for the batch experiment was already adapted to that temperature. During the incubation period, a stable thermophilic temperature of 54 °C was maintained by external circulator. The small temperature fluctuation of ±1 °C occurred mainly when fresh water was added in order to compensate water losses due to evaporation.

During anaerobic digestion, temperature setting is an important factor, as at different temperature ranges the microbial consortia are different. Temperature stability is also an important operating parameter as methanogenic bacteria are more sensitive to temperature changes than other microorganisms. Most microbial consortia can tolerate only small changes in temperature when they have adapted to a certain temperature without change of their composition. Thus, a change of the temperature leads to a drastic alteration of the growth rate of the microorganisms.

In contrast, in this study the aerobic test was carried-out at ambient temperature of 25 °C. The temperature was ranged between 27 and 23.5 °C. At this temperature range, nitrification can take place, which will enable aerobic degradation of nitrogen-rich organic matter (see **Fig. 4.20** and **Fig. 4.21**). The optimum temperature for nitrification, a key step in the aerobic digestion process, was reported by several authors to be 30-35 °C (Neufeld et al., 1986; Antoniou et al., 1990; Willers et al., 2002). However, Zupančič and Roš (2008) reported that the nitrification was performed well in the range of 22 to 30 °C. According to their results, the nitrification rate decreased from 30 to 35 °C and was totally inhibited at 38 °C.

pH-value

The course of the pH during the anaerobic and aerobic batch test is shown in **Fig. 4.17**. In the anaerobic batch test, the pH value increased from 7.13 to 7.87 during the first 4 d of incubation, and then stabilized at 7.4 ± 0.1 for the remaining incubation period. The pH increase at the beginning was caused by ammonification, where organic nitrogen is transformed to ammonium, which was shown by the increase of the ammonia concentration from 1.2 to 1.6 g/kg f.w. (see: paragraph "Nitrogen", **Fig. 4.20**).

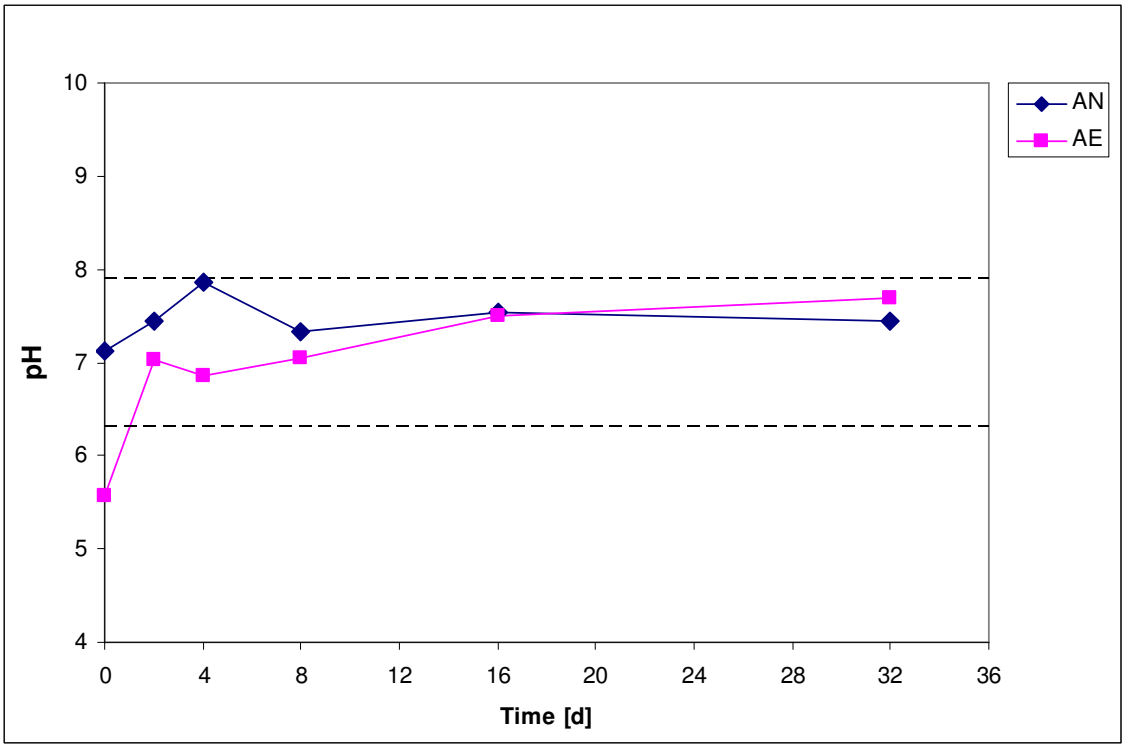


Figure 4.17: Dynamics of the pH during anaerobic (AN) and aerobic (AE) batch test. The dash lines represent the recommended pH range for methanogenic condition

For the anaerobic process, the pH of the system and its stability is an important parameter to maintain methanogenic conditions. In general, methanogenic bacteria function optimally in the neutral to slightly basic range. At a pH <6.3 and >7.8, the methanogenesis rate tends to decline (El-Mashad et al., 2004). A lower pH inhibits methanogens while a higher pH increases the concentration of unionized ammonia, causing toxicity in the reactor (Lay et al., 1997). When methanogenesis is decreased, acids will accumulate, and further decreasing the pH lead to the termination of methane production.

During the aerobic batch test, a higher change of the pH value was observed (**Fig. 4.17**) as in a case of the anaerobic digestion. During the first 2 d of incubation, the pH value sharply

increased from 5.57 to 7.03, slightly decreased again to 6.85 until day 4, and then slightly increased up to pH 7.70 until the end of incubation period. A low pH at the beginning of incubation is expected as the raw sludge used as inoculum was acidic (pH = 5.4). However, the pH during the whole experiment was in a comfortable range for aerobic digestion.

Redox potential (E_h)

In the anaerobic batch test, the E_h gradually decreased from -40 to -180 mV after 32 d. (**Fig. 4.18**). According to Stumm and Morgan (1996), methanogenic conditions are characterized by an E_h of -200 mV and E_h range of -200 to 200 mV is indicated for anoxic conditions. Even though the E_h was > -200 mV during the whole test, the detection of CH_4 production from the beginning of the test (see: paragraph "Biogas", **Fig. 4.22**) showed that methanogenic conditions were given.

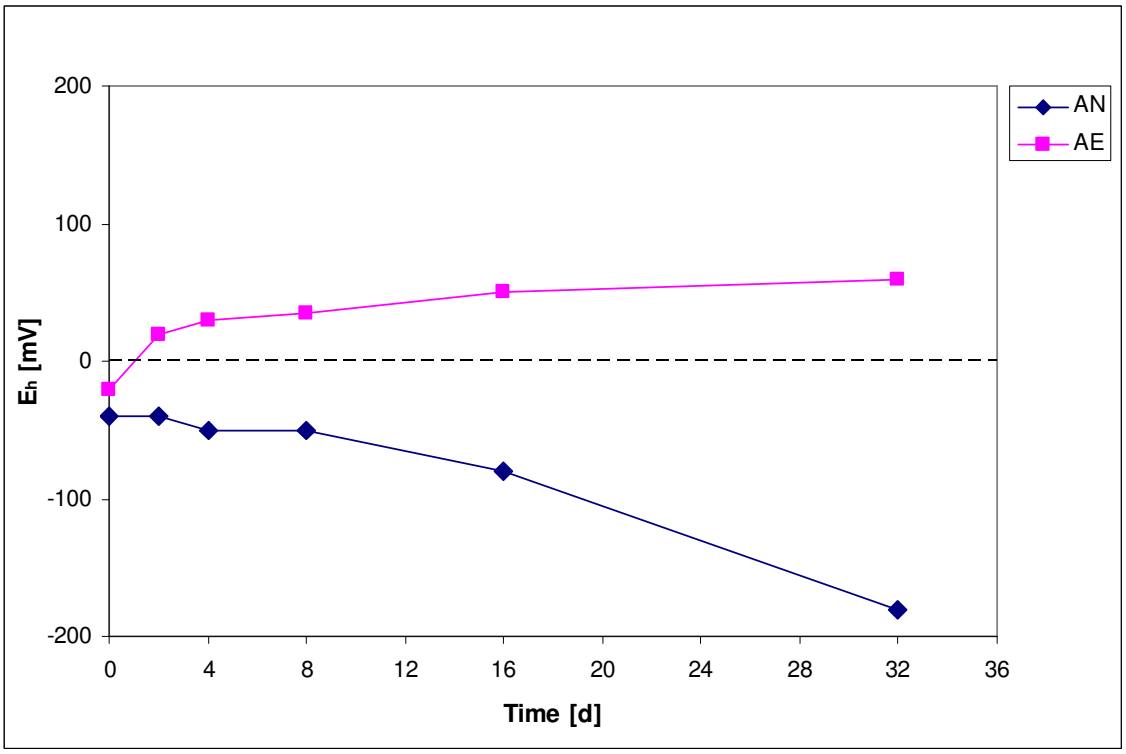


Figure 4.18: Dynamics of the redox potential (E_h) [mV] during anaerobic (AN) and aerobic (AE) batch test

Anaerobic incubation has created reducing zones in the test system which can vary from nitrate-reducing to methanogenic. Methanogenic conditions can be identified based on biogas production while nitrate-reducing conditions enable ammonification (nitrate → ammonium) and denitrification (nitrate → N_2).

In contrast, the aerobic batch test was characterized by mainly positive E_h values, ranging from -20 mV at day 0 to 60 mV at day 32, as an effect of intensive aeration. It is known that an intensive aeration system is necessary for aerobic digestion to promote the aerobic degradation (Ponti et al., 1995; Messenger et al., 1993). Accordingly, the measured dissolved oxygen (DO) concentrations during the aerobic batch test were in the range 0.3 to 0.6 mg/L, which was ≤7% of saturated conditions (9 mg/L). The reason was a very rapid consumption of the introduced oxygen by the microorganisms due to the high amount of available carbon in the sludge. However, the measurement of the DO concentration showed that a slight amount of oxygen was always available. According to Stumm and Morgan (1996), real aerobic conditions are achieved at E_h >200 mV. In this study, "real" aerobic conditions were not achieved, but anoxic conditions were established.

TOC

During anaerobic batch test, an 50% increase of TOC was observed from 11 to 17 g/kg f.w. (**Fig. 4.19**). In parallel, the d.s. increased 17% from 2.65 to 3.09%. The increase of TOC and d.s. resulted from feeding raw sludge every 3 d during anaerobic batch process in order to maintain the CH_4 production.

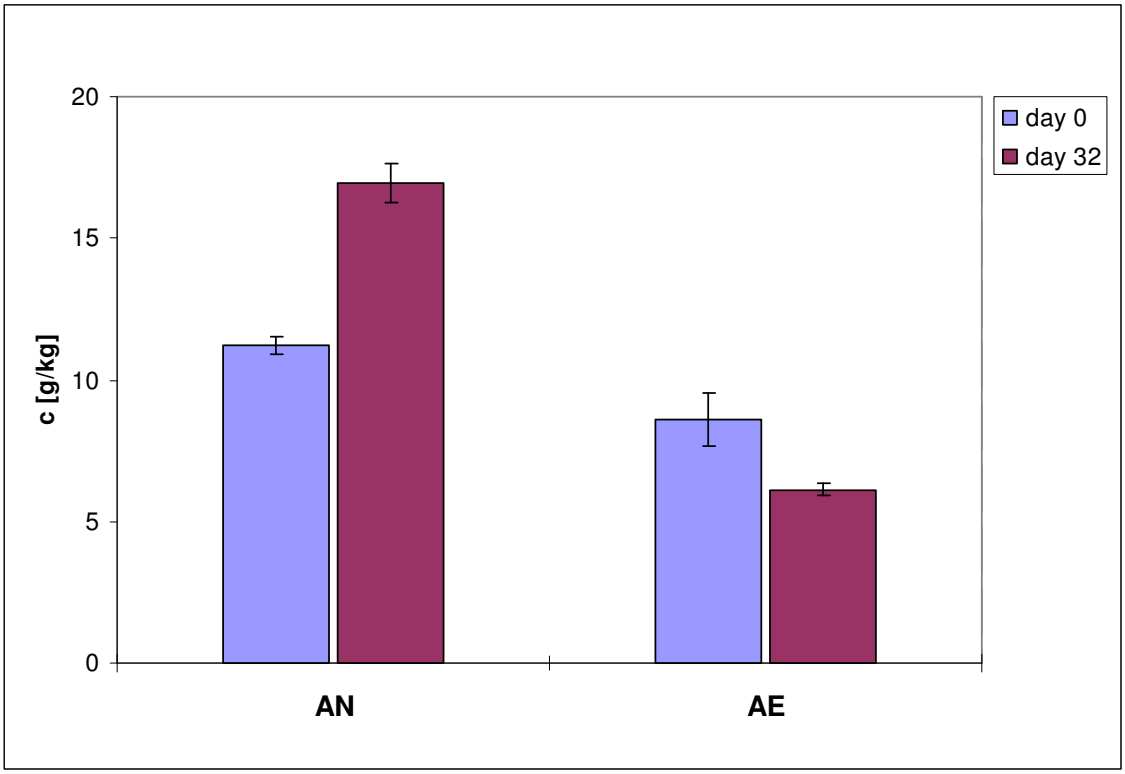


Figure 4.19: Organic carbon (TOC) dynamics during removal during anaerobic (AN) and aerobic (AE) batch test, n = 2

In contrast, TOC and d.s. reduction rates during aerobic incubation were 29 and 32%, respectively (**Fig. 4.19**). At the aerobic batch test, aerobic heterotrophic microorganisms degraded available organic material and no additional raw sludge was fed during the the test period. About 50% of the organic material which is consumed by the microorganisms is used for the synthesis of new cells, resulting in a biomass increase (Zupančič and Ros, 2008). However, the other 50% are consumed for energy production and thus cause in total loss of carbon by the release of CO_2.

Nitrogen

Nitrogen was determined as total Kjehldal nitrogen (TKN), ammonium, nitrate and nitrite. TKN and ammonium nitrogen were analysed in total sludge, and nitrate and nitrite only in the liquid phase of the sludge. An increase of TKN and ammonia during anaerobic batch test was observed (**Fig. 4.20**). In the beginning of the incubation, the TKN was approximately 2.22 g/kg f.w. and the ammonia content was 1.22 g/kg f.w. which was equivalent to 55% of the TKN. After incubation for 32 d, TKN increased to 2.56 g/kg f.w. and the ammonia content increased to 1.71 g/kg f.w. which was equivalent to 67% of the TKN. This result showed that organic nitrogen was not removed but partly converted to ammonia.

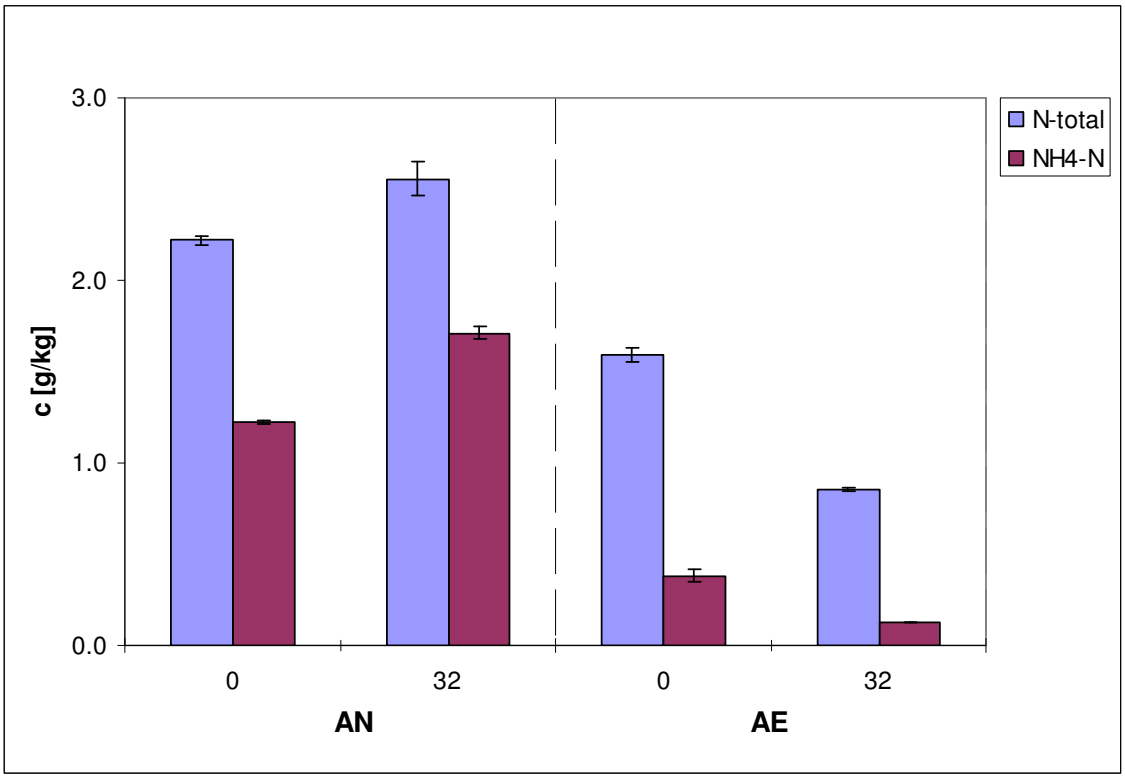


Figure 4.20: Nitrogen (TKN and ammonia) dynamics during anaerobic (AN) and aerobic (AE) batch test, n = 2. The value of pH of both processes was at the same level (pH ~7)

In the anaerobic batch test, the ammonia concentration of 1.71 g/kg f.w. was reached (**Fig. 4.20**). The generation of ammonia (ammonification) is an indicator that the methanogenic process was stable. Ammonia concentration <200 mg/kg were reported to be beneficial since nitrogen is an essential nutrient for anaerobic microorganisms (Chen et al., 2008). However, it is known that ammonia concentrations >200 mg/kg is toxic for methanogenic bacteria and thus can inhibit the production of methane (Tada et al., 2005). As the pK_a value of ammonia is 9.24 at pH ≤7.2, the ratio of NH_4^+/NH_3 will be 100 (2 pH rule), which means most ammonia will be present in the form of NH_4^+, so that total ammonia concentrations up to 3 g/kg can be tolerated (Novak et al., 2011). In contrast, at pH values >8.2, relevant amount of free ammonia (NH_3) will be formed and this free ammonia will affect the methanogenic digestion by inhibiting the enzyme or by penetrating into the cells and causing a proton imbalance (Kayhanian, 1999). In this study, pH was maintained ≤7.7, thus most ammonia (30 times) was not converted to NH_3 and the concentration of NH_3 should always be <50 mg/kg (calculated with Henderson-Hasselbach equation).

During aerobic batch test, a significant removal of TKN and ammonia was observed (**Fig. 4.21**).

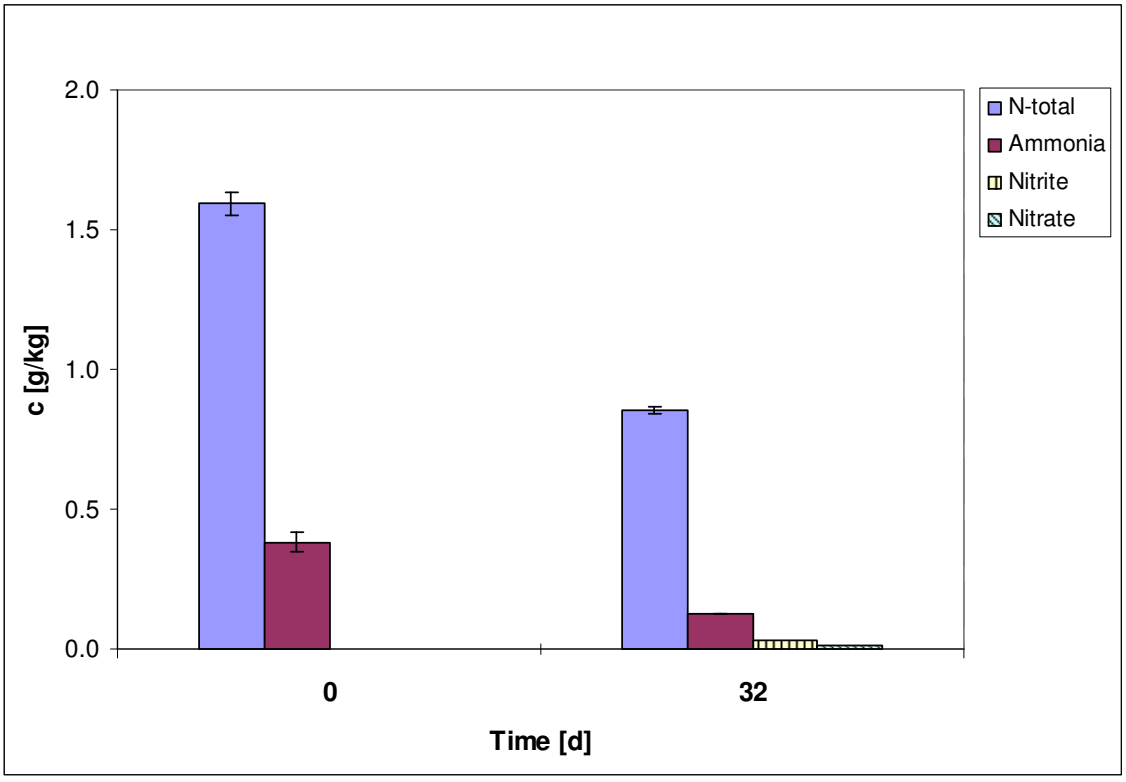


Figure 4.21: Nitrite and nitrate formation during aerobic (AE) batch test, n = 1; TKN, ammonia n = 2

At the beginning of the aerobic incubation, the TKN was 1.59 g/kg f.w. and the ammonia content was 0.38 g/kg f.w., which was equivalent to 24% of the TKN. After incubation for 32 d, TKN decreased to 0.85 g/kg f.w. and the ammonia content in parallel decreased to 0.13 g/kg, equivalent to 15% of the TKN. Furthermore, 30 mg/kg f.w. nitrite and 10 mg/kg f.w. nitrate was detected after 32 d. The finding of TKN removal and high nitrite concentration in relation to nitrate reflects that the conditions in the aerobic batch were not really aerobic (compare **Fig. 4.18**). However, the TKN removal and nitrite formation indicates a denitrification process that was shown by a total loss of 46% of nitrogen during the digestion. The reason might be an insufficient aeration in the test system. As the aeration was performed by diffuser stones (compare **Fig. 3.5**), the introduced air might be not distributed over the whole flask volume. Hence, the peripheral zones were not fully aerated causing anaerobic zones.

Biogas production

Methanogenesis was confirmed by the measurement of biogas production (CH_4 and CO_2), which was continuously monitored during the anaerobic batch experiments. Daily and cumulative biogas productions are shown in **Fig. 4.22**. Biogas production was observed already immediately after incubation was started. After the initial lag phase (about 4 d), increasing of biogas production was observed. In total, biogas production increased until day 29, reaching a total biogas volume 1500 mL. As the next stage, the plateau phase with nearly no further gas production was observed. The curve of daily biogas production showed a jawtooth-cycle that correspond to the feeding cycle of the batch test. Following the feeding with 3 g of raw sludge, an increase of biogas production was observed. However, after 1 d, the biogas production was back to decrease.

In order to proof that the produced gas was truly a biogas, semi-quantitative gas analyses were performed during eight sampling times. CH_4 concentration ranged from 10 to 28% v/v proofing that methanogenesis took place. However, the CH_4 yield was relatively low compared to values from literature. The normal range for CH_4 was reported to be 50-70% and 30-40% for CO_2 (e.g. Sefeedpari et al., 2012). The reason for the differences to reported values from literatures was perhaps the biogas sampling and the injection technique (described in **Ch. 3.4.10**, **Fig. 3.10**) which caused dilution of CH_4 by ambient air. This dilution might occur mainly during biogas collection from volumetric cylinder to sampling syringe, considering the large space of the rubber hose and the connector tube containing air.

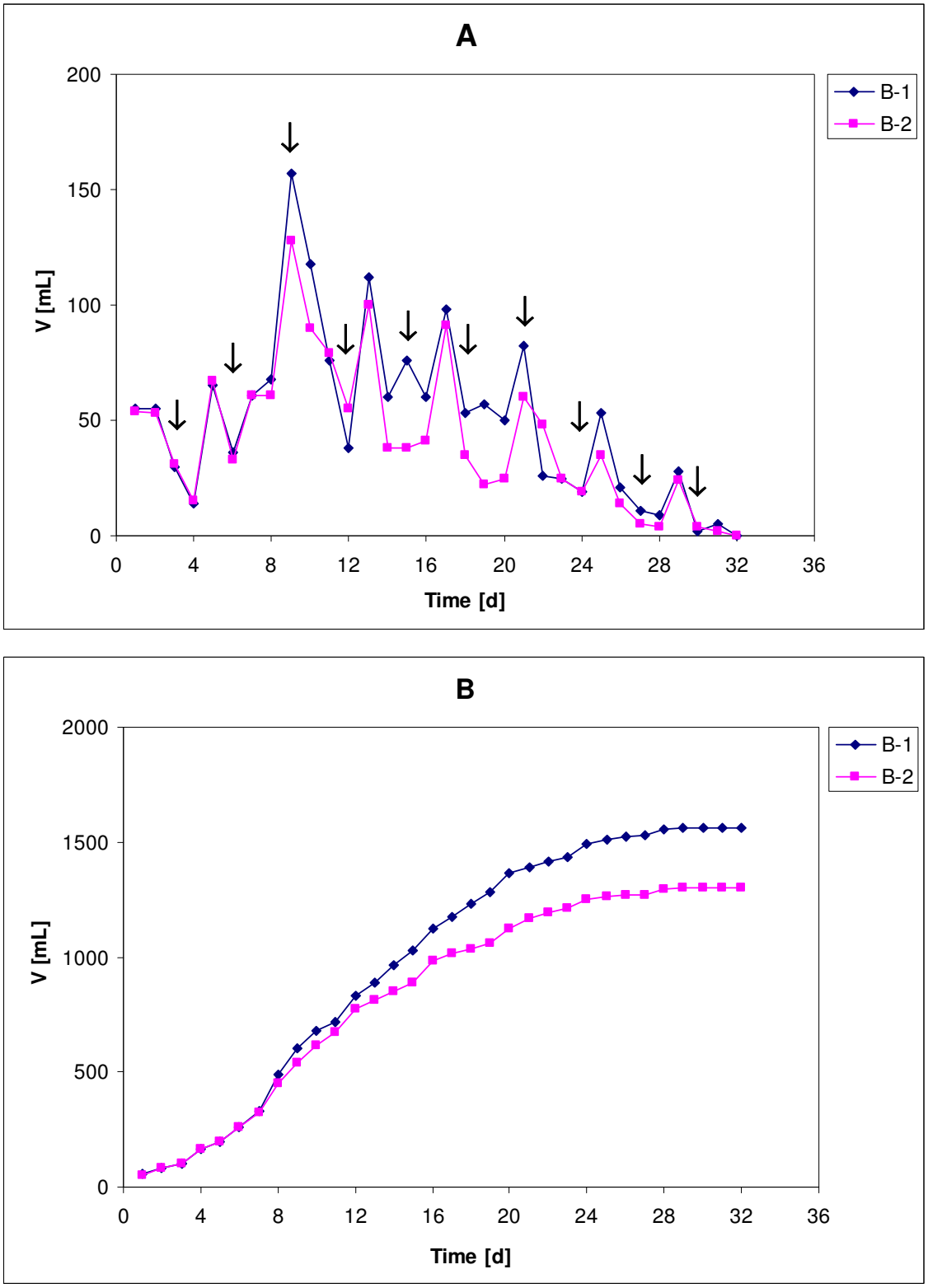


Figure 4.22: Daily (**A**) and cumulative (**B**) biogas production during anaerobic batch test. Data obtained from two unspiked (blank) samples (B-1 and B-2); ↓ = feeding by raw sludge

4.2.3 Dissipation of BFRs in the batch test

In this study, the term of “dissipation” was used when the concentration of the parent compound was decreasing. Because just by considering the decrease of the concentration, the processes of changes like degradation, transformation, or sorption, could not be distinguished. For each sampling date, the concentration of the test compounds was determined, and the change of their concentration was calculated.

The spiked concentration level of the BFRs test compounds in the batch test was chosen considering the background concentration of test compounds in the sewage sludge matrices from WWTP of Braunschweig and the average range of BFRs in sludge samples of other European WWTP obtained from literature as summarized in **Tab. 4.8**. All selected test compounds were detected in the raw sludge of WWTP of Braunschweig. The presence of deBDethane at a concentration of 57 µg/kg d.s., which was 40% of BDE-209, was unexpected as this compound is relatively new on the EU market. TBBPA and HBCD were found at lower concentrations 16 and 7 µg/kg d.s., respectively. In total, the test compounds were present at low µg/kg d.s. range, confirming data from the literature, which were reported for sludges from WWTP in Germany and other European countries (**Ch. 1.3.3**, **Tab. 1.3**). The spiking level for batch experiments were 350 to 36,000-fold higher than the measured background concentration, in a mg/kg range. Thus, the interference of the background concentration during the batch experiment was negligible.

Table 4.8: BFRs concentration range in European sludge, background concentration, spiked level, and concentration ratio

Test compounds	Conc. range of European WWTP[1] [µg/kg d.s.]	Background concentration[2] [µg/kg d.s.]	Batch tests spiked level [µg/kg d.s.]	Concentration ratio
TBBPA	0.3 - 1,329	16	50,000	3,000
HBCD	0.6 - 9,120	7	250,000	36,000
BDE-209	0.2 - 9,411	142	50,000	350
deBDethane	0.2 - 257	57	25,000	440

[1]Data obtained from WWTP in Germany, Czech, Ireland, Italy, Netherlands, Spain, Sweden, Switzerland, and UK; [2]Measured in the raw sludge from WWTP of Braunschweig (n = 4)

Anaerobic batch test

During the anaerobic batch test (AN set-up), HBCD was eliminated most rapid. In the first 2 d, HBCD concentration decreased already to 26%, and at day 4, the concentration was already <LOD. The dissipation rates for TBBPA and BDE-209 were obviously slower. In the first 4 d, still 90% of TBBA was detected. However, after 16 d, just 4% of TBBPA was detected and after 32 d, the concentration was already <LOD, whereas during the same period 30% of BDE-209 was still detected. DeBDethane was not removed in the anaerobic batch test, and only small changes in the range of 95 to 106% (RSD = 4.30%) of the initial concentration were observed. These changes were only a little bigger than the analytical error (RSD = ±3%).

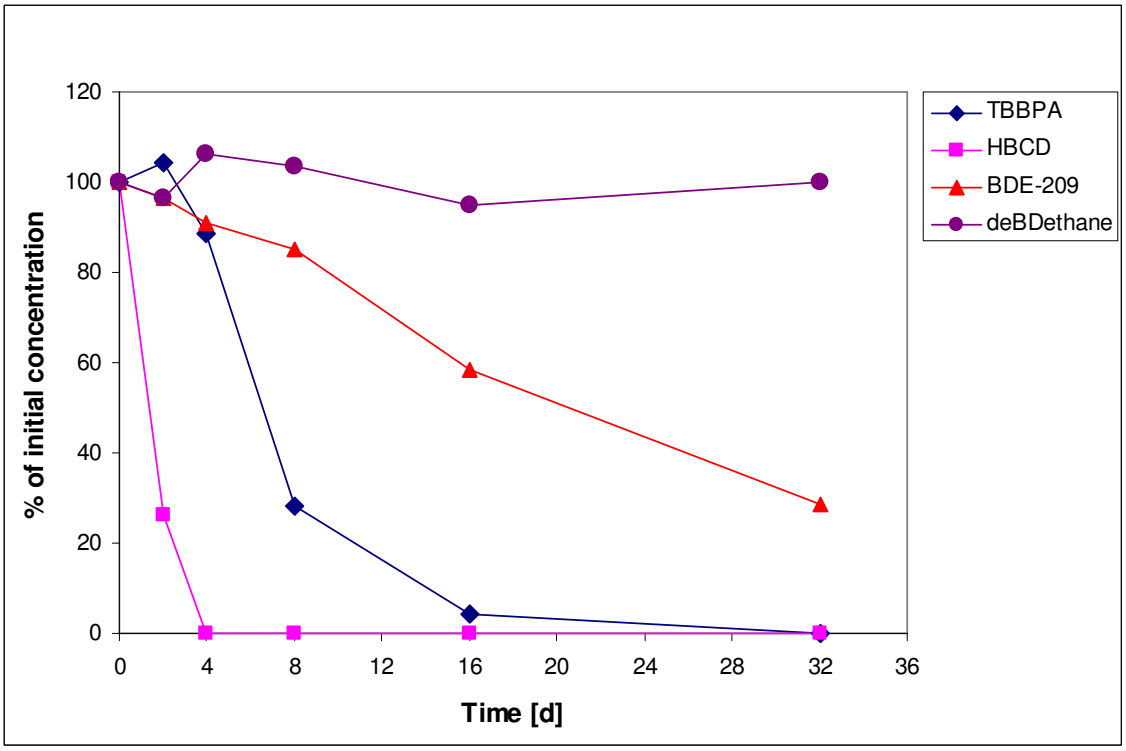


Figure 4.23: Dissipation profile (remaining concentration [%] versus time [d]) of BFRs under anaerobic batch test (AN set-up), T = 54 °C

The calculation of dissipation kinetics of test compounds from plots of the natural logarithm of the concentration [% of initial concentration] versus time [d] is shown in **Fig. 4.24**. For HBCD, the pseudo-first-order dissipation rate constant (k) was 0.68 d^{-1}, corresponding to a DT_{50} of 1.0 d., whereas the k for TBBPA and BDE-209 were obviously lower, 0.20 d^{-1} corresponding to a DT_{50} of 3.5 d and 0.04 d^{-1} corresponding to a DT_{50} of 17 d, respectively. For deBDethane, a k-value of 0.0005 d^{-1} and DT_{50} of >1300 d were determined. Thus, under

anaerobic conditions the order of dissipation was as follow: HBCD > TBBPA > BDE-209 >> deBDethane.

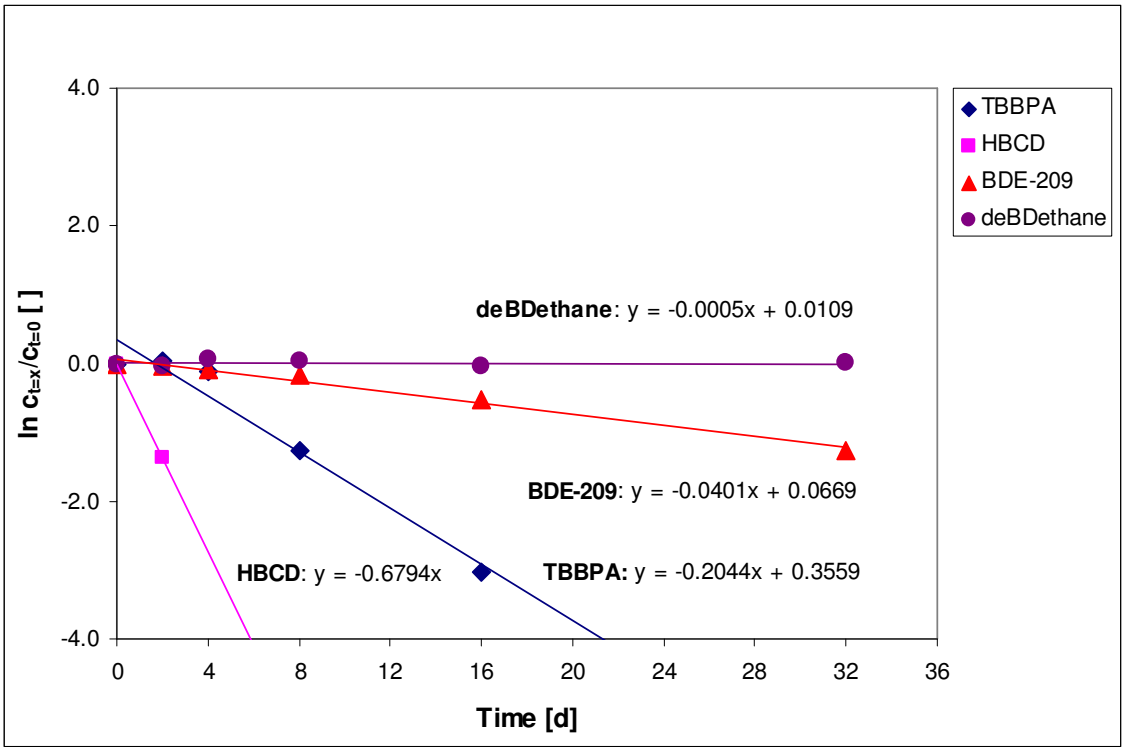


Figure 4.24: Dissipation profile (**ln $c_{(t=x)}/c_{(t=0)}$ [ ]** vs time [d]) of BFRs in sludge under anaerobic batch test (AN set-up)

The current study can be compared with the result from Gerecke et al. (2006) who performed a laboratory-scale degradation test for several BFRs compounds (TBBPA, HBCD, and BDE-209) under anaerobic conditions. The experiment was carried out in 300 mL glass serum bottles, with a digested sewage sludge taken from Dübendorf WWTP, Switzerland. The batch tests were incubated under mesophilic conditions at 37 ± 1 °C for 238 d. For the other different experimental aspects, some primers, chemically related compounds that have been reported to stimulate reductive dehalogenation, including 2,6-dibromobiphenyl, 4-bromobenzoic acid, and decabromobiphenyl, were included in the Gerecke's experiment. Starch and yeast were also added as nutrients.

For HBCD, a similar result was reported by Gerecke et al. (2006) who showed a rapid degradation of the technical HBCD with a pseudo-first-order rate constant of 1.1 d^{-1}, corresponding to a DT_{50} of 0.66 d (**Tab. 4.9**). Thus, Gerecke et al. reported a little faster HBCD dissipation rate than observed in this study, by the factor of 1.5. This indicated that

thermophilic and mesophilic condition did not produce significant difference for the dissipation of HBCD. Furthermore, the degradation rate of HBCD was not dependent on the presence of additional nutrients or primers. Davis et al. (2005) reported an anaerobic biotransformation of HBCD in anaerobic soil and two different sediments from Schuylkill River, PA, USA and Neshaminy Creek, PA, USA (**Tab. 4.9**). Rapid degradation of HBCD was observed, which correspond to DT_{50} of 6.9 d for anaerobic soil and 1.5 and 1.1 d for Schuylkill River and Neshaminy Creek sediments, respectively.

Table 4.9: Data of dissipation kinetic constants (k, [d^{-1}]) and half-live (DT_{50}, [d]) of the test compounds under anaerobic conditions from literature

Test compounds	Medium	k [d^{-1}]	DT_{50} [d]	References
HBCD	Sludge	1.1 ± 0.3	0.6	Gerecke et al. (2006)
	Sludge	-	15	Davis et al. (2006)
	Sediment	0.45	1.5	Davis et al. (2005)
	Sediment	0.61	1.1	Davis et al. (2005)
	Soil	0.10	6.9	Davis et al. (2005)
	Sludge	**0.68**	**1.0**	current study
TBBPA	Sludge	1.2 ± 0.06	0.6	Gerecke et al. (2006)
	Sediment	1.3 ± 0.09	0.5	Ronen and Abeliovich (2000)
	Sludge	-	ND	Brenner et al. (2006)
	Sludge	**0.20**	**3.5**	current study
BDE-209	Sludge	0.001	700	Gerecke et al. (2006)
	Sediment	-	15	Parsons et al. (2004)
	Sludge	**0.04**	**17**	current study
deBDethane	Sludge	**0.0005**	**>1000**	current study

k: pseudo-first-order dissipation rate constant [d^{-1}]; DT_{50}: the time required for 50% dissipation of the compound [d]; ND: no dissipation was observed

In the study of Gerecke et al. (2006), TBBPA was reported to have a DT_{50} of 0.59 d, which was 6 times faster than the DT_{50} obtained in this study (**Tab. 4.9**). As in case of HBCD, no obvious difference on the dissipation rate of TBBPA under thermophilic (DT_{50} = 3.5 d) and under mesophilic conditions (DT_{50} = 0.6 d). However, Brenner et al., (2006) investigated the degradation of TBBPA in lab-scale anaerobic reactors with sludge as a matrix. In this study, no TBBPA degradation was detected. For sediments, Ronen and Abeliovich (2000) reported

DT_{50} of 0.5 d for TBBPA under anaerobic conditions. Biotransformation of TBBPA in estuarine sediments was also reported by Voordeckers et al. (2002). They observed a complete dehalogenation of TBBPA to BPA under both methanogenic and sulfate-reducing conditions. Forthermore, under methanogenic condition, a nearly complete loss of TBBPA was found within 55 d.

In case of BDE-209, Gerecke et al. (2006) reported a much slower dissipation rate for BDE-209 (DT_{50} of 700 d) in digested anaerobic sewage sludge than found in this study (DT_{50} of 17 d) (**Tab. 4.9**). The current study was performed under thermophilic condition (54 °C), compared to mesophilic condition (37 °C) in the study of Gerecke et al. In the current study, the initial BDE-209 concentration was about 3-fold higher than the concentration reported by Gerecke et al. In Gerecke et al., spiking concentration of 16 mg/kg d.s. was applied, while in this study was 50 mg/kg d.s. Furthermore, in the current study, a raw sludge was added as nutrient instead of starch and yeast. These aspects are perhaps the reasons for the differences. Batch test study with anaerobic soil by Nyholm et al. (2010) also showed no significant degradation of BDE-209 (DT_{50} >390 d) during the incubation period for 160 d. However, Parsons et al. (2004) reported about a faster removal of BDE-209 in anaerobic sediment (DT_{50} = ~15 d), where marine sediment samples from industrial area of Western Scheldt, Netherlands, were used as inoculum. The sediments were spiked with BDE-209 (14 mg/kg sediment) and anaerobically incubated at room temperature in the dark. A decrease in the concentration of BDE-209 was observed, where approximately 70% removal of BDE-209 was obtained during the first 25 d of the experiment. Overall, the current study and the study of Parsons et al. show that BDE-209 might be not completely persistent in an anaerobic environment and the formation of lower brominated congeners could proof this possible degradation.

The calculated k of deBDethane was 0.0005 d^{-1}, corresponding to a DT50 of >1300 d (**Tab. 4.9**), which means that nearly no change of the concentration was obtained. The big differences between the dissipation rate of deBDethane and BDE-209 were not expected due to their structural similarity. However, due to the ethane bridge between the two fully brominated aromatic rings instead of an ether bridge, deBDethane has a little bigger molecular size and is more lipophilic (P_{ow} = >7) than BDE-209 (P_{ow} = 6.27). Therefore, bioavailability of deBDethane is assumed to be very limited. This aspect might be a rate limiting factor for enzymatically mediated transformation. So far, no data about the anaerobic incubation of deBDethane were reported in the literature.

Aerobic batch test

The dissipation of BFRs was also evaluated in an aerobic batch test (AE set-up). In comparison to anaerobic batch test, BFRs showed a different behaviour in the aerobic test (**Fig. 4.25**).

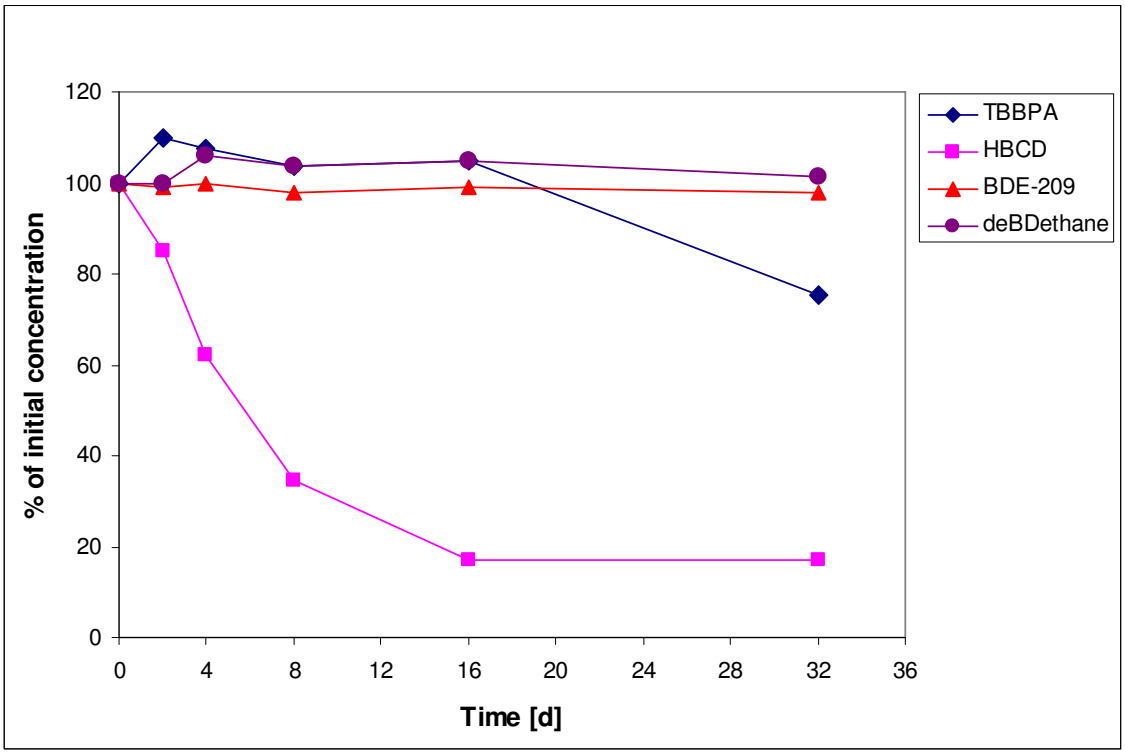


Figure 4.25: Dissipation profile (remaining concentration [%] versus time [d]) of BFRs under aerobic batch test (AE set-up)

HBCD decreased less rapidly, and only 17% was still detected after 16 d. A much slower dissipation rate than in the anaerobic test was observed for TBBPA. Up to 16 d, no concentration decrease of TBBPA was observed. However, this lag period was followed by an apparent decrease of TBBPA to 75% after 32 d. In case of BDE-209, no obvious decrease of the concentration was obtained during 32 d. The concentration ranged between 98% and 100% of the initial concentration. Similar to the anaerobic conditions, deBDetane was also not removed under aeration. The concentration ranged between 100 to 105% from the initial concentration.

The calculation of dissipation kinetics of test compounds under aerobic conditions from plots of the natural logarithm of the concentration [% of initial concentration] versus time [d] is shown in **Fig. 4.26**. The calculated k-values showed the order of dissipation as follow: HBCD >> TBBPA > BDE-209 ≅ deBDethane.

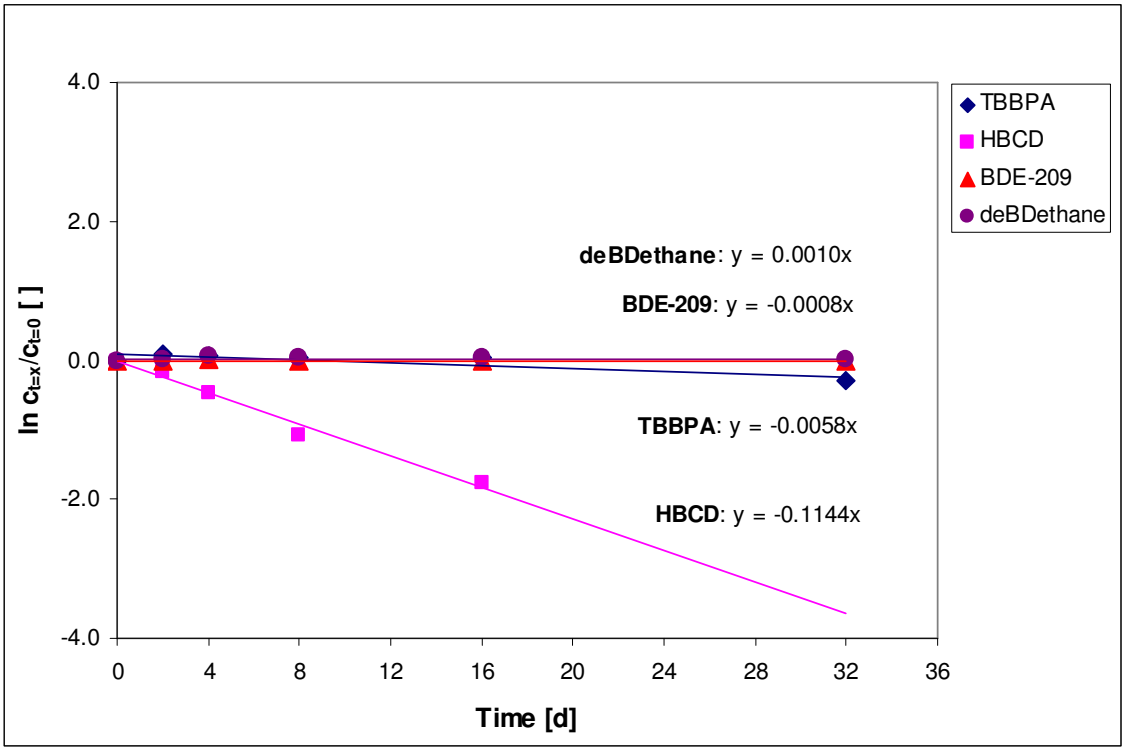


Figure 4.26: Dissipation profile (**ln $c_{(t=x)}/c_{(t=0)}$ [ ]** vs time [d]) of BFRs in sludge under aerobic conditions (AE set-up)

The k-value of HBCD was 0.11 d^{-1}, corresponding to a DT_{50} of 6.3 d., which was 6-fold lower than in the anaerobic batch test. Davis et al. (2005) investigated the dissipation of HBCD in aerobic soil and sediments. In their study, aerobic soils and sediment samples were collected from surface layer 15 cm depth of soil and 0-5 cm depth of sediment. Batch tests were prepared in 250 mL serum bottles and were continuously exposed with ambient air to create aerobic conditions (E_h = -18 to 151 mV). The bottles were incubated in the dark at 21 °C for 49 d. A DT_{50} of 63 d was reported HBCD in soil and of 11 and 32 d in Schuylkill River and Neshaminy Creek sediments, respectively (**Tab. 4.10**).

The k-value of TBBPA was 0.0058 d^{-1}, corresponding to a DT_{50} of 120 d. This result was 34-fold slower than TBBPA dissipation under anaerobic conditions. Brenner et al. (2006) conducted an aerobic degradation test of TBBPA in a lab-scale reactor fed with sludge and together with contaminated sediments that were supposed to have indigenous bacteria exposed to the compound. However, no TBBPA dissipation and no accumulation of intermediates such as BPA were detected (**Tab. 4.10**). Nyholm et al. (2010) reported a DT_{50} values for TBBPA in aerobic soil amended with sewage sludge that were in a similar range as the results of this study. Surface soil (a heavy clay agricultural soil) and sewage sludges

(activated and digested sludges) were used in aerobic batch tests in amber bottles with caps that enabled an air exchange. The bottles were stored at room temperature (20 °C) for up to 160 d. The mean DT_{50} of TBBPA was 65 d (58-75 d) in soil amended with activated sludge and 93 d (78-110 d) in soil amended with digested sludge.

Table 4.10: Data of dissipation kinetic constants (k, [d^{-1}]) and half-live (DT_{50}, [d]) of the test compounds under aerobic conditions from literature

Test compounds	Medium	k [d^{-1}]	DT_{50} [d]	References
HBCD	Sediment	0.022	32	Davis et al. (2005)
	Sediment	0.066	11	Davis et al. (2005)
	Soil	0.011	63	Davis et al. (2005)
	Sludge	**0.11**	**6.3**	current study
TBBPA	Soil+activated sludge	-	65	Nyholm et al. (2010)
	Soil+digested sludge	-	93	Nyholm et al. (2010)
	Sludge	-	ND	Brenner et al. (2006)
	Sludge	**0.0058**	**120**	current study
BDE-209	Soil+activated sludge	-	>400	Nyholm et al. (2010)
	Soil+digested sludge	-	>360	Nyholm et al. (2010)
	Sludge	**0.0008**	>**800**	current study
deBDethane	Sludge	**0.0010**	>**1000**	current study

k: pseudo-first-order dissipation rate constant [d^{-1}]; DT_{50}: the time required for 50% dissipation of the compound [d]; ND: no dissipation was observed

As during aerobic incubation nearly no decrease of BDE-209 was observed, the k-value was 0.0008 d^{-1}, corresponding to a DT_{50} of 866 d. This result is consistent with results of Nyholm et al. (2010) who found that BDE-209 showed no significant degradation under aerobic condition during the entire incubation period of 160 d in aerobic soil amended with activated and digested sludge (**Tab. 4.10**). A DT_{50} of >400 and >360 were determined, respectively. However, Stiborová et al. (2008) found a degradation of PBDEs by the indigenous microflora

under aerobic conditions. For BDE-209, the authors reported a decrease of 20%. The author did not calculate dissipation kinetics and DT_{50} value.

As already in the anaerobic test, deBDethane showed persistence in the aerobic batch test. By calculation, a very small k-value of 0.0010 d^{-1} was obtained, which was comparable to the result from the anaerobic test. So far, no data for deBDethane were reported in the literature.

Anaerobic-aerobic batch test

This test was performed in order to evaluate the effect of sequential anaerobic and aerobic batch test (AN-AE set-up) on the dissipation of the test compounds. The behaviour of BFRs under sequential anaerobic-aerobic treatments is shown in **Fig. 4.27**.

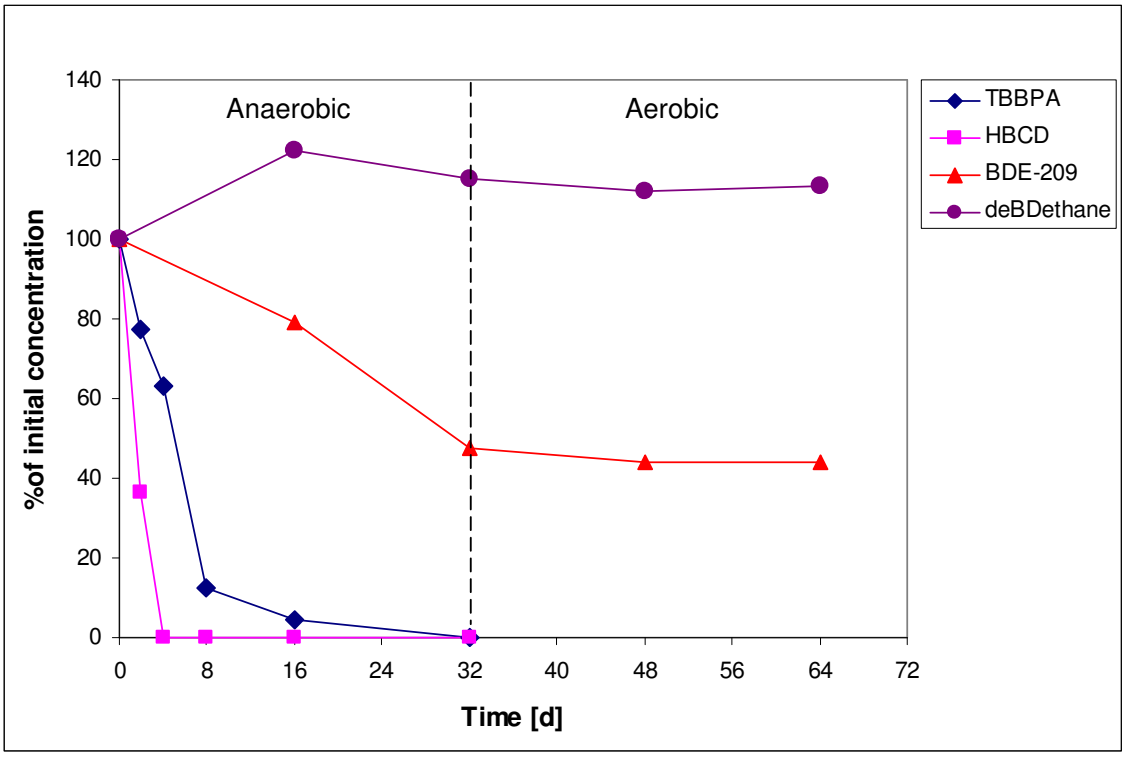


Figure 4.27: Dissipation profile (remaining concentration [%] versus time [d]) of BDE-209 in anaerobic-aerobic incubation (AN-AE set-up)

HBCD and TBBPA showed a relative rapid dissipation in the anaerobic batch test. Thus, both compounds could not be detected anymore after 32 d. Nearly the same results were observed during the sequential anaerobic-aerobic batch test. The result showed a good reproducibility of the batch tests for these two compounds, also with sludges from different sampling dates.

In the sequential anaerobic-aerobic batch test, BDE-209 was also rapidly dissipated during the anaerobic step. However, the dissipation rate was found a little bit lower than in the single anaerobic experiment. After 32 d incubation, still 48% of BDE-209 was detected, while only 29% was detected during single anaerobic test. The reason of this different result might be a different composition of the used sludges in this experiment, where sludge "B" was applied for single anaerobic test and sludge "C" for sequential anaerobic-aerobic test. As they were taken at different sampling date, the activity of the microbial community could be different. From matrices characterization, several parameters were found different between both sludges, such as d.s., TOC, and TKN (**Ch. 4.2.1**, **Tab. 4.7**). During the subsequent aerobic step, BDE-209 concentration was only slighly dissipated. At the end of the aerobic step, still 44% of BDE-209 was detected, which corresponded to a further decrease of 4%. This value is comparable with the result from the aerobic experiment, where only small decrease of 2% of BDE-209 was observed during 32 d.

Similar to BDE-209, no removal of deBDethane was observed in the sequential anaerobic-aerobic batch test during 62 d of incubation (**Fig. 4.28**). However, the total concentration range of 100 to 122% was higher compared to the single aerobic experiment with a range of 100 to 106%. The calculated dissipation rate constant of test compounds during anaerobic-aerobic incubation and was summarized in Tab**. 4.11**.

Table 4.11: Dissipation kinetics of BFRs in three different batch test set-up

Test compounds	k [d^{-1}]			DT_{50} [d]		
	AN	**AE**	**AN-AE**	**AN**	**AE**	**AN-AE**
TBBPA	0.20	0.0058	0.20	3.5	120	3.5
HBCD	0.68	0.11	0.51	1.0	6.3	1.3
BDE-209	0.04	0.0008	0.02	17	>800	35
DeBDethane	0.0005	0.0010	0.0011	>1000	>1000	>1000

AN: anaerobic batch test; AE: aerobic batch test; AN-AE: anaerobic-aerobic batch test; k: pseudo-first-order dissipation rate constant [d^{-1}]; DT_{50}: the time required for 50% dissipation of the compound [d]

The results show that only the anaerobic treatment is effective in order to reduce the concentration of TBBPA, HBCD, and BDE-209. Under aerobic treatment, the reduction is obviously lower, especially in case of more persistent compounds, TBBPA and BDE-209. In case of less persistent compounds HBCD, the difference is less. The molecules of TBBPA

and BDE-209 are highly brominated aromatic rings, which have to be debrominated in the first step of microbial degradation. It is known from literature that for such dehalogenation of aromatic rings, anaerobic conditions are needed (e.g. Segev et al., 2009). In case of the aerobic batch test, the conditions were not clearly in the aerobic range (E_h = -20 to +60 mV, compare: paragraph "Redox potential", **Fig. 4.18**). Thus, it is assumed that also a low percentage of anaerobic dehalogenation took place, which led to the slight decrease of TBBPA and BDE-209 also in the aerobic batch test. In case of HBCD, the molecule has an aliphatic ring structure which is not totally brominated. Therefore, also under aerobic conditions the ring can be attacked by microorganisms.

UV/Vis irradiation batch test

Drying process of raw sludge under sunlight as performed in many arid countries was simulated in a batch test with UV/Vis radiation at 30 °C. For this purpose, a thin layer of sludge was radiated for 20 d, 12 h/d in order to simulate day and night cycle. A control experiment was performed under dark conditions. During the incubation period, the temperature of the dark experiment was also maintained at 30 °C. The behaviour of the test compounds under dark experiment is shown in **Fig. 4.28**.

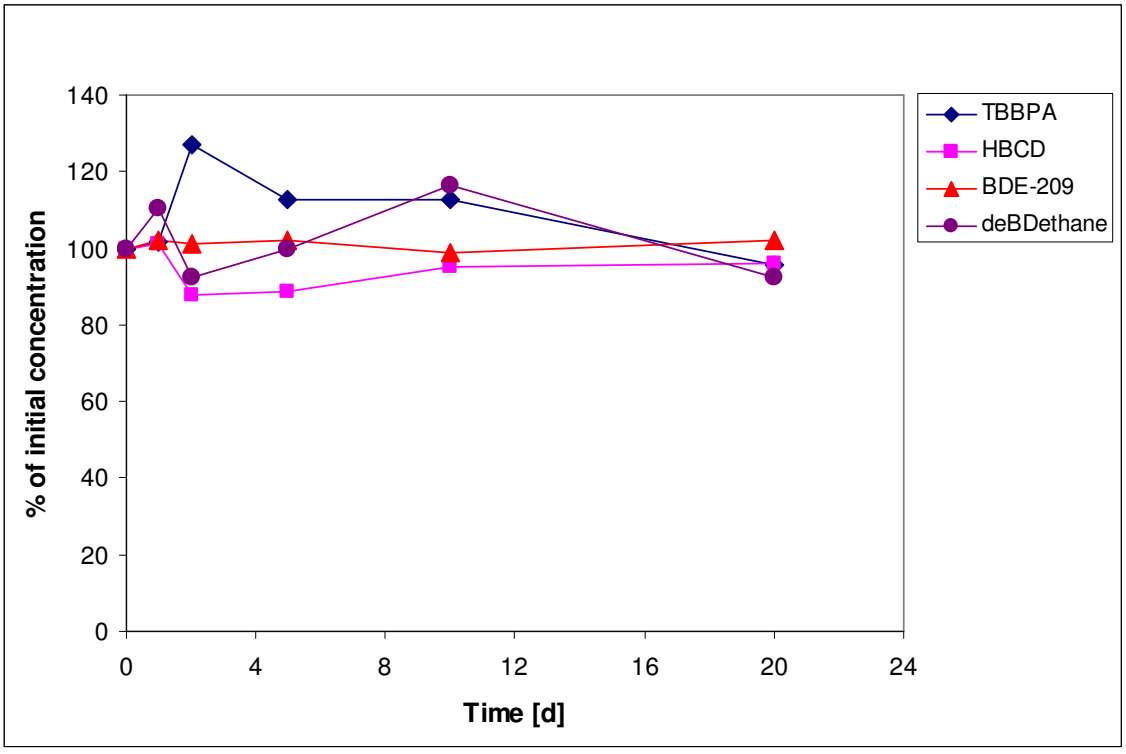


Figure 4.28: Dissipation profile (remaining concentration [%] versus time [d]) of BFRs in the dark experiment

In the dark experiment, the concentrations of the test compounds were nearly stable, ranging from 93 to 116 %, except a relatively high recovery (127%), which was measured for TBBPA at day 2. These variations of the recovery rates were in the range of the recovery rates from fortification experiments. Therefore, they are assumed to be caused by the variability of the analytical method. Overall, the dark experiment showed that the concentration of the test compounds was not decreased by evaporation under the experimental conditions. Despite the findings, that HBCD was fast dissipated after 1 d in the aerobic conditions, no dissipation was observed in the dark experiment. The explanation of this condition was a low water content (d.s. >94%) in the sludge matrix after drying for 24 h that stops the microbial activity.

Under UV/Vis irradiation, different dissipation profile of the test compounds is shown in **Fig. 4.29**.

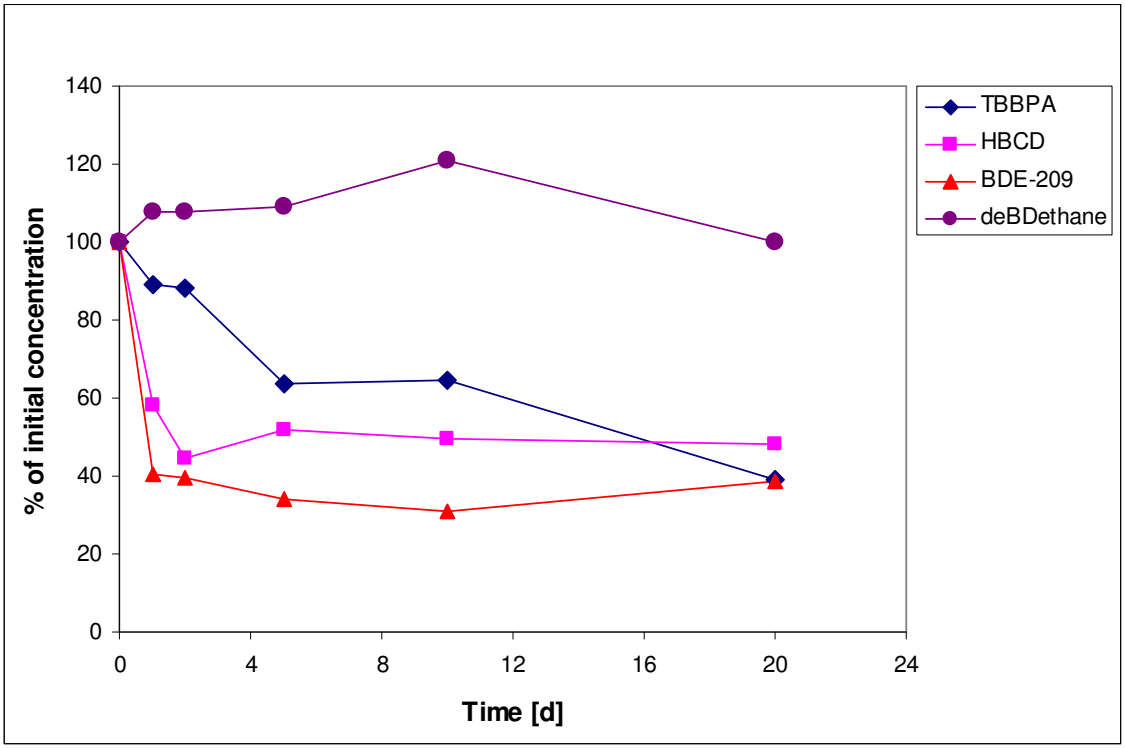


Figure 4.29: Dissipation profile (remaining concentration [%] versus time [d]) of BFRs under UV/Vis irradiated batch test (LI set-up)

The concentration of HBCD and BDE-209 decreased very rapidly. In case of BDE-209, the concentration decreased after 1 d to 40%, and in case of HBCD within 2 d to 44%. However, a further dissipation of the compound was not observed until the end of the experiment (20 d). Due to the dark colour of the sludge, light energy cannot penetrate into deeper layers of the sludge. Thus, dissipation did only occur on the surface layer. A continuous mixing of the

sludge during irradiation would allow the irradiation of all parts of the sludge and thus a more efficient removal of the BFRs. A gradual disappearance of TBBPA was observed in the light experiment. During the first 2 d, only a slight decrease to 88% had occurred. A subsequent loss to 65% was measured after 5 d, and a further decrease to 40% was observed until the end of the experiment. In contrast to the rapid dissipation of BDE-209, surprisingly no removal of deBDethane was observed during UV/Vis irradiation.

The dissipation kinetics of the test compounds under UV/Vis irradiation is shown in **Fig. 4.31**. In case of HBCD and BDE-209, only the data until day 3 were used for the calculation as the concentrations afterwards were not changing anymore. This has to be considered for the application of the calculated equations and constants. They are only applicable for a limited time range (0 to 2 d). The calculated k values showed the order of dissipation as follows: BDE-209 ≅ HBCD > TBBPA >> deBDethane.

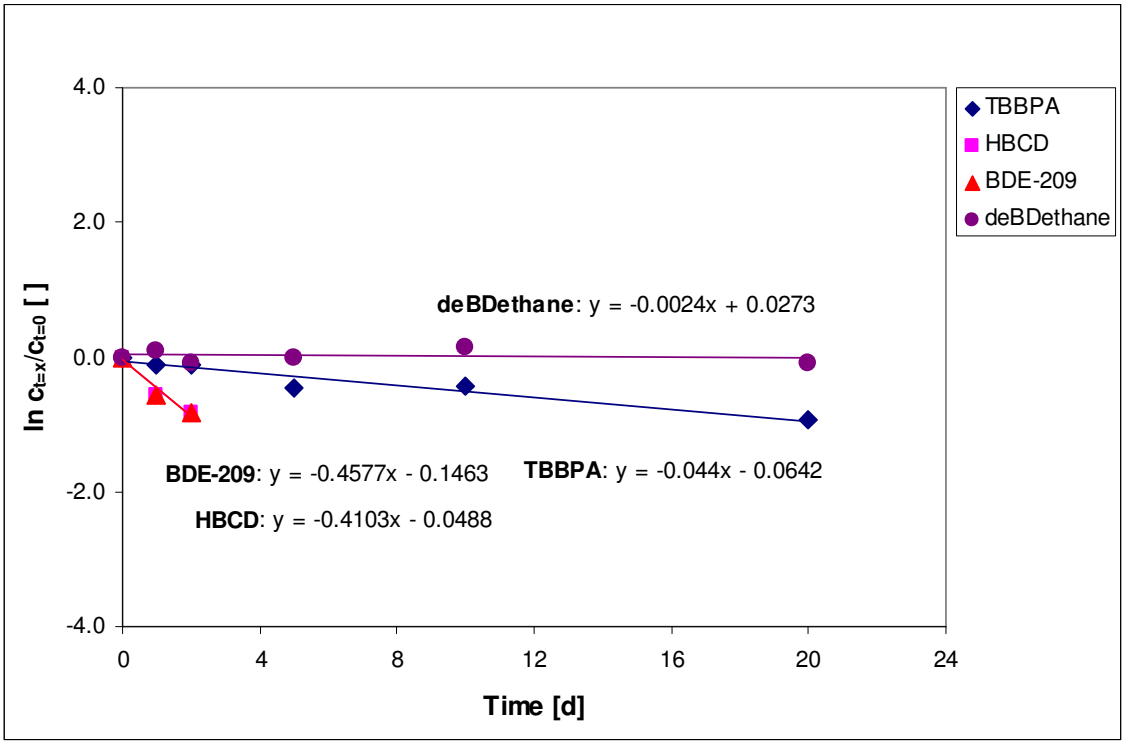


Figure 4.30: Dissipation profile (**ln $c_{(t=x)}/c_{(t=0)}$ [ ]** vs time [d]) of BFRs under UV/Vis irradiated batch test (LI set-up)

Those dissipation data cannot be fully interpreted based on the UV absorption profile of the test compounds. In general, the absorptivity of the test compounds decreased in parallel with the increase of wavelength higher than 290 nm (λ lamp cut-off) (**Fig. 4.31**). The highest response was shown by TBBPA, which has an absorption maximum at 295 nm. Lower

response (approximately by the factor of half) was shown by BDE-209 with an absorption maximum at 310 nm. Furthermore, a much lower response was shown by deBDethane and HBCD in that wavelength region.

Rapid dissipation of HBCD was thus surprising. The calculated k-value was 0.41 d^{-1}, corresponding to a DT_{50} of 1.7 d. It is assumed that fast dissipation of HBCD observed in the current study might be as a result of the aerobic condition of the sludge matrices. However, an obvious removal of HBCD was not observed in the dark experiment of HBCD.

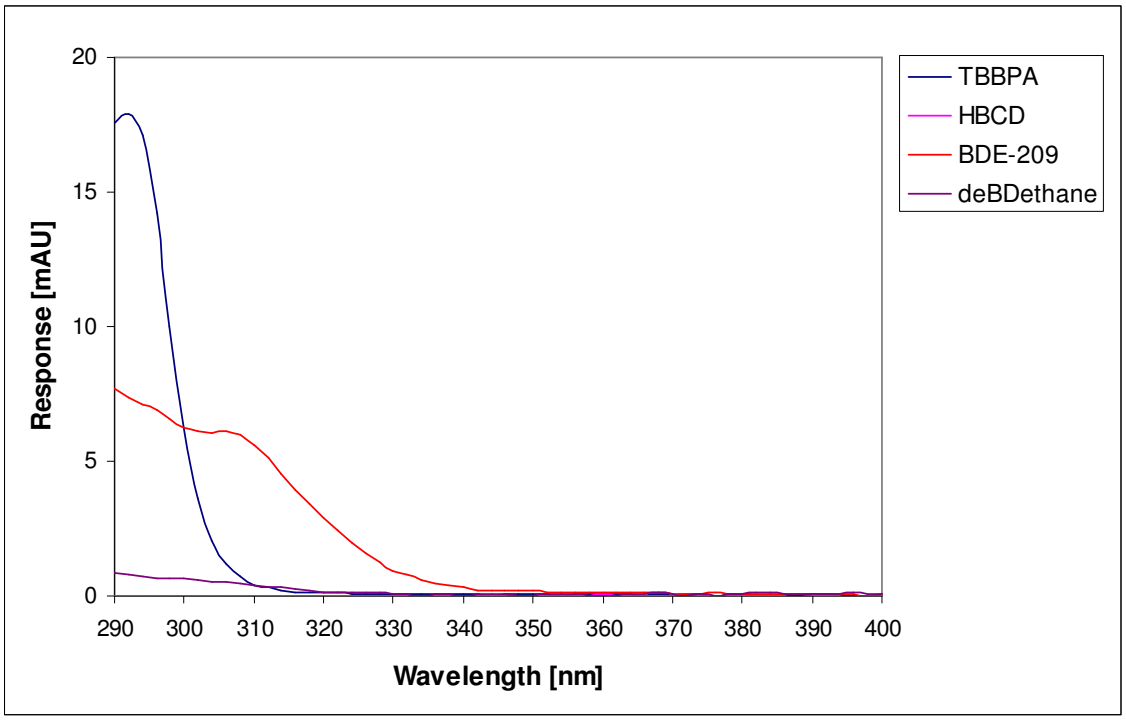


Figure 4.31: Absorption spectrum of test compounds in methanol (λ = 290-400 nm). TBBPA (50 ng/uL); HBCD (250 ng/uL); BDE-209 (50 ng/uL); deBDethane (25 ng/uL)

Rapid dissipation of BDE-209 observed in the current study was in parallel with the high UV absorption in the wavelength range of 290-400 nm. The calculated k-value was 0.46 d^{-1}, corresponding to a DT_{50} of 1.5 d. Previous photolysis studies of BDE-209 focused mainly on the in various matrices (i.e. sand, sediments, soils, etc.) with artificial UV lamps or sunlight as irradiation sources (**Tab. 4.12**). For example, the half-lives of BDE-209 sorbed on clay minerals (such as montmorillonite and kaolinite) was reported as 36 and 44 d, respectively (Ahn et al., 2006). Kajiwara et al. (2008) reported photolysis of BDE-209 in a polymer (HIPS) under natural sunlight with a DT_{50} of 51 d.

The current results show that photolytic debromination of BDE-209 is an important pathway for the dissipation of BDE-209. A free radical mechanism has been proposed to explain the photolysis process, i.e. via homolytic breaking of the halogen and/or ether bonds of PBDEs, generating aryl and bromine radicals (Watanabe and Tatsukawa, 1987; Rayne et al., 2006; Suh et al., 2009). Söderström et al. (2004) found that degradation rates are slower in the natural matrices compared to those in the artificial materials. Furthermore, the presence of organic matter, e.g. humic acids, was found to significantly reduce the photolysis process (Hua et al., 2003).

Table 4.12: Dissipation kinetic (k, [d^{-1}]) and half-live (DT_{50}, [d]) of the test compounds under UV/Vis irradiated batch test from literature

Test compounds	Medium	k [d^{-1}]	DT_{50} [d]	References
BDE-209	Montmorillonite	-	36	Ahn et al. (2005)
	Kaolinite	-	44	Ahn et al. (2005)
	Soil	-	6.3-8.3	Söderström et al. (2004)
	Sediment	-	1.6-2.5	Söderström et al. (2004)
	Sediment	-	150	Ahn et al. (2005)
	Indoor Dust	-	17*	Stapleton and Dodder (2008)
	HIPS	-	127*	Kajiwara, et al. (2008)
	Sludge	**0.46**	**1.5**	current study
HBCD	Indoor dust	-	85*	Harrad, et al. (2009)
	Sludge	**0.41**	**1.7**	current study
TBBPA	Water (pH = 7)	-	1.7	Eriksson et al. (2004b)
	Sludge	**0.04**	**15.7**	current study
deBDethane	HIPS	-	>1000	Kajiwara et al. (2008)
	Sludge	**0.0004**	**>1000**	current study

k = pseudo-first-order dissipation rate constant [d^{-1}]; DT_{50} = the time required for 50% dissipation of the compound [d]; *experiment under sunlight radiation

In contrast to the rapid dissipation of BDE-209, deBDethane was resistant to UV/Vis treatment in sludge. The calculated k-value was 0.0004 d^{-1}, corresponding to a DT_{50} of >1000 d. So far, no studies dealing with UV/Vis radiation of deBDethane in sewage sludge matrices are published. However, deBDethane photolytic degradation in different liquid and solid matrices was reported. Wang et al. (2012) investigated photodegradation of this compound

in different solvents (n-hexane, THF, methanol/water) and other matrices (humic acid/water, and silica gel). They found a rapid dissipation (<320 min) of deBDethane. A similar degradation of BDE-209 and deBDethane during photodegradation experiments supported by carboxylates under visible lights was observed by other authors (Sun et al., 2013). The comparable results with the current study were reported by Kajiwara et al. (2008) in the photodebromination study of deBDethane incorporated into plastics polymer (HIPS) and TV casings under natural sunlight. There was no marked loss of deBDethane observed throughout the experimental period of 224 d.

4.2.4 Formation of degradation products

The current study demonstrates BFRs dissipation under anaerobic, aerobic, and UV/Vis irradiation. However, in order to proof degradation of BFRs, the samples were also analyzed for degradation products, mainly lower brominated transformation products. GC/MS chromatograms of BDE-209 under anaerobic conditions showed the presence of the group of peaks, which were identified as degradation products of BDE-209 (**Fig. 4.32**).

At the day 0, only peak BDE-209 was observed (t_R = 48.61 min). At day 16, two groups of peaks were observed. The first group (9 Br) consists of 2 peaks (t_R = 31.89 and 32.76 min) with a higher intensity. The second group (8 Br) consists of 3 peaks (t_R = 23.72, 24.16, and 24.59 min) with a lower intensity were also observed. After 32 d, the peak intensity of the second group was significantly increased. Other two small peaks were also observed. Meanwhile, the peak intensity of the first group remained constant. The formation of those degradation products was accompanied by the reduction of the intensity of the parent compound peak. These peaks identity has not been reported so far due to the absence of authentic standards. However, it was predicted as nona-BDE (9 Br) and octa-BDE (8 Br), and other lower brominated congeners.

The mass spectrum of BDE-209 and the degradation intermediates observed during the anaerobic and UV/Vis irradiation batch test are given in **Fig. 4.33**. The molecular ions [M^+] for these peaks were obtained as m/z 859.4, 881.4 and 799.6, suggesting the formation of nona- and octa-BDE congeners. The most abundant fragment ions for BDE-209, nona-, and octa-BDE were at m/z 799.5, 719.5, and 641.6, respectively, corresponding to the ion fragment of $[C_{12}H_1Br_8O]^+$, $[C_{12}H_1Br_7O]^+$, and $[C_{12}H_1Br_6O]^+$. Further formation of lower brominated BDE congeners (tri to hepta-BDE) was expected to occur. These results indicate that the first step of the degradation of BDE-209 is the loss of one bromine atom to form nona-BDE congeners and subsequent another one bromine atom loss to form octa-BDEs.

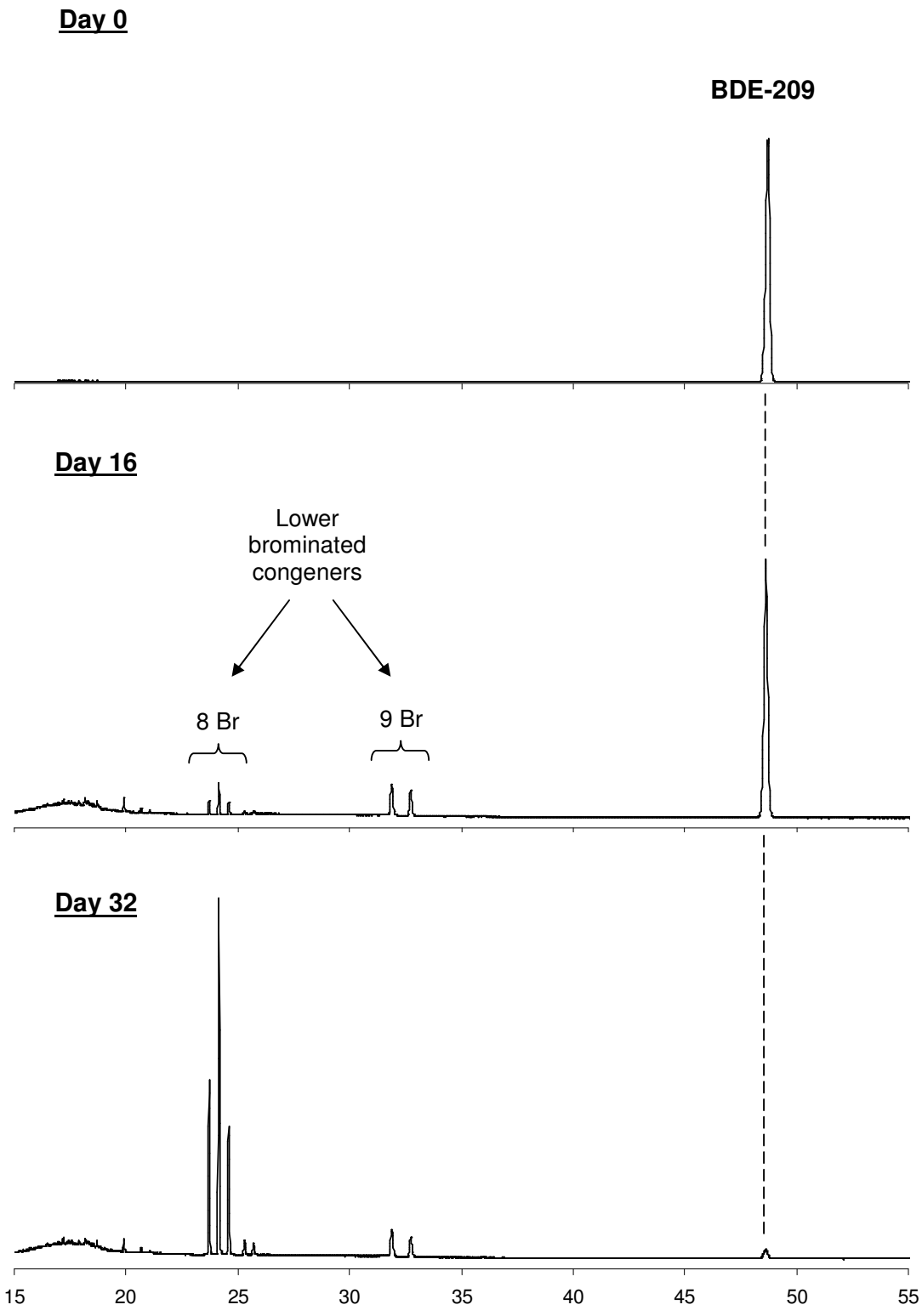


Figure 4.32: GC/MS chromatogram (EI, full-scan mode) shows of sludge extract during anaerobic batch test of sludge spiked with BDE-209

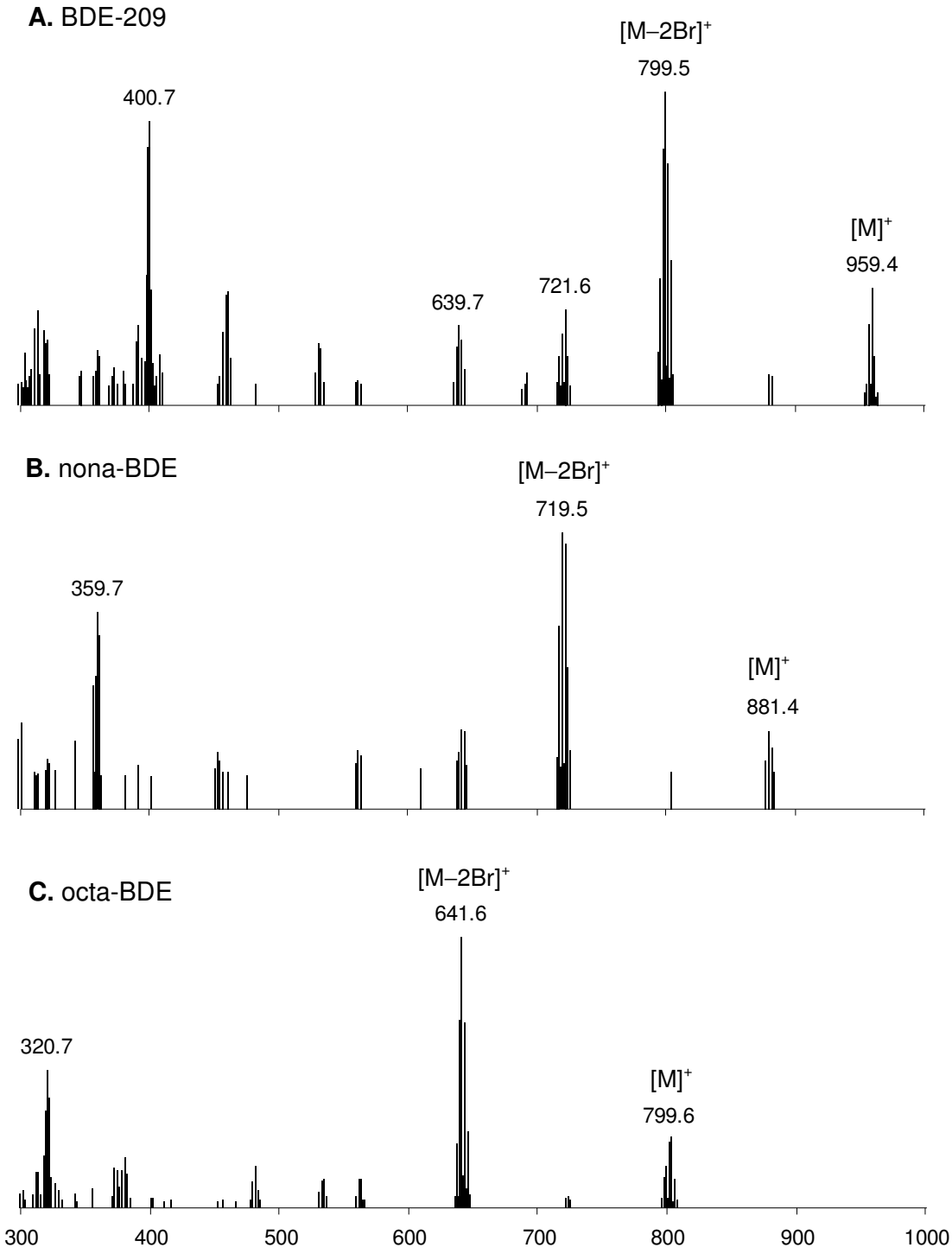


Figure 4.33: Mass spectra (EI; full-scan mode) of BDE-209 (MW = 959.2) (**A**) during anaerobic incubation showed a formation of lower brominated congeners, nona-BDE (MW = 880.3) (**B**) and octa-BDE (MW = 801.4) (**C**)

As the comparison, the formation of lower brominated BFRs were not detected in GC/MS chromatogram of BDE-209 under aerobic incubation (**Fig. 4.34**) which does not facilitate the degradation of BDE-209. GC/MS chromatogram showed no reduction of parent compounds peak intensity during the incubation time (32 d).

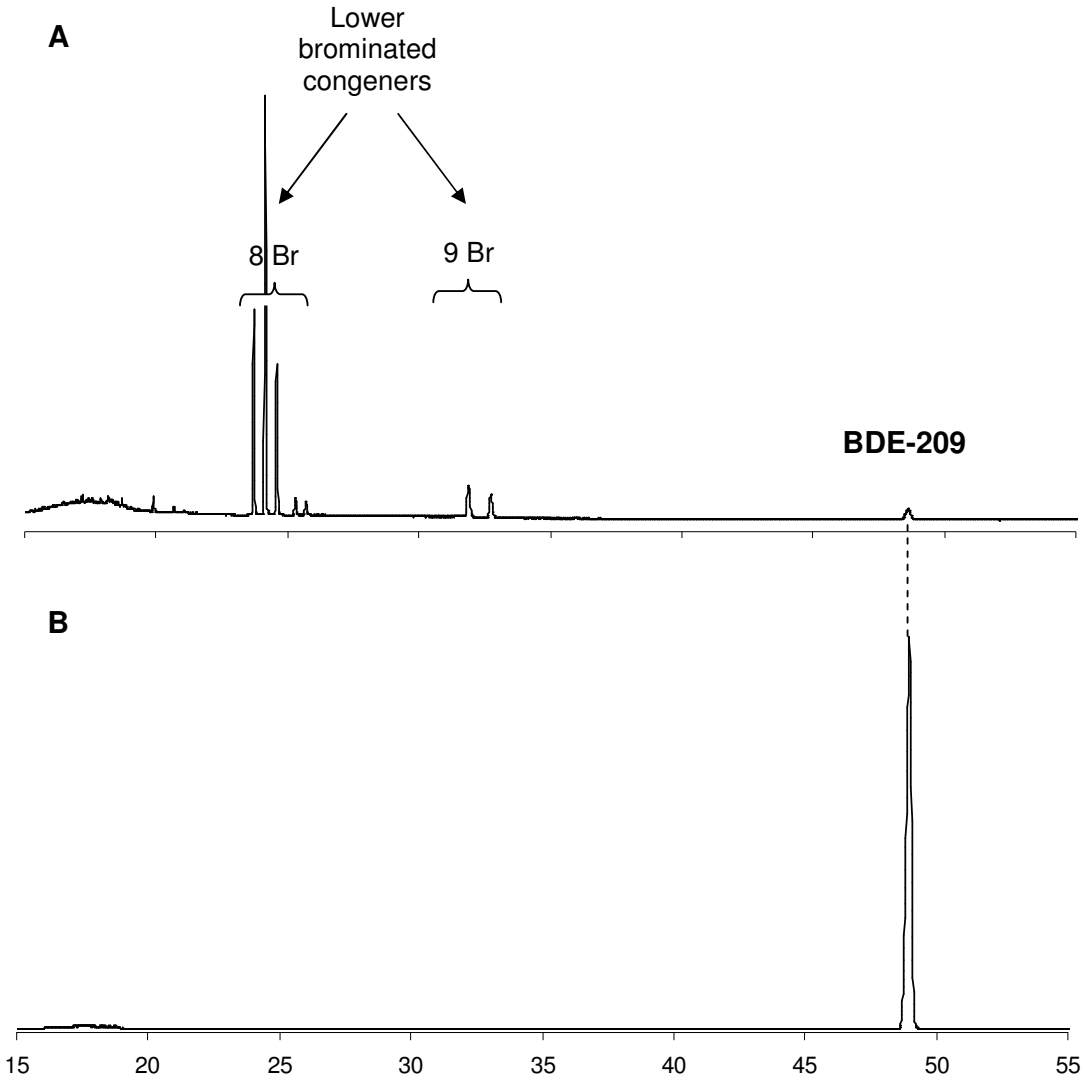


Figure 4.34: GC/MS chromatogram (EI, full-scan mode) of BDE-209 shows the absence of lower brominated congeners formations during aerobic batch test (**B**) after 32 d incubation, in comparison to those in anaerobic batch test (**A**)

These results confirm the literature findings on the reductive dehalogenation of BFRs under anaerobic conditions. In such reactions, BFRs serve as electron acceptors in respiratory or cometabolic processes. Two mechanisms were proposed for the dehalogenation reaction

under anaerobic conditions, which are reduction and hydrolysis (Sims, et al., 1991). Reductive mechanisms are recognized as the predominant pathway for removal of halogens from homocyclic aromatic rings, as BDE-209 and deBDethane. In contrast to BDE-209, deBDethane showed resistance to biodegradation. It is perhaps caused by their bulky molecular size (MW >900 g/mol), resulting in the uptake delay into cells and enzymatic attack during biodegradation. Furthermore, these similar patterns of sequential debromination of BDE-209 were also observed during UV/Vis irradiation batch test, suggesting that BDE-209 adsorb UV light and then undergo sequential bromine losses.

5. Conclusions

The fate and behaviour of selected BFR compounds were studied in a simulated sludge treatment by different batch test systems: aerobic, anaerobic, sequential anaerobic and aerobic, and UV/Vis irradiation.

The analysis of BFRs in a complex matrix such as sewage sludge needs comprehensive approaches. In this study, Soxhlet extraction with a mixture of hexane/acetone (1:1, v/v) for 16 h was chosen as extraction method and followed by multi-step cleanup method before instrumental analysis. GPC was chosen as a first cleanup step for organic matter as well as elemental sulphur elimination. For TBBPA and HBCD, a further multi-layer column, consist of neutral and acidified silica gel, was applied as the second cleanup step. HPLC/DAD was optimized for the quantification of the test compounds. In addition, GC/MS analysis was performed for identification of possible degradation products.

From the batch experiment, with the exception for deBDethane, all the test compounds were eliminated at the highest rate under anaerobic condition. Thus, it could be concluded that anaerobic conditions cause the most efficient dissipation of BFRs from sewage sludge while BFRs are removed less efficiently under aeration. In case of HBCD, the substance was dissipated to a large amount under anaerobic as well as under more aerobic conditions. However, the rapid dissipation of HBCD under UV/Vis irradiation might be more a result of a transformation process under aerobic condition. TBBPA can be dissipated under anaerobic as well as under aerobic conditions. However, the dissipation rate is slower than for HBCD. A combination of anaerobic treatment and drying under irradiation might be advantageous, especially for a more effective degradation of BDE-209. However, debromination of BDE-209 can produce another environmental problem considering the formation of more toxic characteristic of the lower bromination congeners of BDE-209.

A further effect of an aeration process after anaerobic incubation cannot be confirmed by the study. However, an aerobic step could be advantageous to enable further demineralization after debromination process of the BFRs compounds, e.g. in case of TBBPA. However, in order to study such degradation in a complex matrix as sewage sludge, the use of 14C-labeled test compounds would be needed. Otherwise, it is not possible to detect debrominated small organic degradation compounds in the diversity of organic matrix molecules.

Considering the persistence criteria by international protocols (UNEP, 2001; EU, 2006), where persistent compounds are classified by half-lives in soil and sediments ranging from 120 to 180 d. The current study showed that not all BFRs have to be classified as persistent in sewage sludge. However, deBDethane, which was introduced into the market in order to substitute BDE-209, showed as a very persistent compound under anaerobic and aerobic conditions and also treatment by UV/Vis irradiation.

6. Summary

Brominated flame retardants (BFRs) are a group of chemicals, which are added to a variety of products to improve their resistance versus fire by inhibiting or suppressing the combustion process. Additive flame retardants, such as BDE-209 and HBCD, are mixed with or dissolved in the material, whereas reactive flame retardants, such as TBBPA, are covalently bound to polymers. BFRs can enter the environment during the production, usage, and disposal of BFR-containing products. Moreover, these chemicals tend to persist in the environment, to accumulate in biota, and to undergo long-range atmospheric transport. This led to increased levels of BFRs in the environment. Wastewater treatment plants (WWTP) are sinks of BFRs, where they enter via domestic or industrial discharges. During WWTP treatment, BFRs tend to be adsorbed into the solids and thus are accumulated in sewage sludge. Therefore, sludge is one main sink of BFRs and further emission to soil or other environmental compartments are possible (i.e. when sludge is used as fertilizer). Detailed knowledge about the fate and behaviour of BFRs during sludge treatments, thus, is important in order to prevent a further distribution of BFRs in the environment. The BFRs (TBBPA, HBCD, BDE-209, and deBDethane), which represent a different groups of BFRs, were chosen as test compounds for batch experiments with sludge.

Prior the batch experiment, an analytical method for test compounds in sludge matrices was developed. A Soxhlet with a mixture of hexane/acetone (1:1, v/v) for 16 h was chosen as extraction method and followed by GPC cleanup. For TBBPA and HBCD, a multilayer silica column was applied as the second cleanup step. HPLC/DAD was optimized for the quantification of the test compounds. In addition, GC/MS analysis was performed for identification of possible degradation products.

In this work, batch experiments of BFRs under different conditions (anaerobic, aerobic, anaerobic-aerobic, and UV/Vis irradiation) were performed in order to simulate different treatment processes of raw sludge as anaerobic digestion, aerobic stabilization, and stabilization by sludge drying with sun irradiation. For the study, raw sludge and digested sludge from WWTP Gut Steinhof of Braunschweig was taken as matrices in the batch experiments. These sludges were spiked with the test compounds before treatment and the concentration profiles during the different treatments were determined. The spiking level was chosen sufficiently high (50 or 250 mg/kg d.s. range), considering the limit of detection by HPLC/DAD, but as low as possible to simulate environmental concentrations. For the anaerobic batch test, thermophilic condition (54 °C) was chosen as the most previous studies were conducted under mesophilic conditions. The aerobic batch tests were performed at 25

°C, and the aerobic regime was created by continuous aeration with air. SRT of anaerobic and aerobic test were 32 d while for UV/Vis irradiation test was 20 d. Different process parameters (i.e. temperature, pH, redox potential (E_h), TOC, nitrogen, for both aerobic and anaerobic batch tests, and biogas production for anaerobic batch test) were continuously monitored and showed that the processes were working well.

In the anaerobic batch test, pseudo-first-order dissipation rate constants decreased according to the following sequence: HBCD > TBBPA > BDE-209 >> deBDethane. DeBDethane was practically not removed under anaerobic conditions (DT_{50} >1000 d) and thus to be classified to be persistent under anaerobic conditions. The results showed that anaerobic condition preferable for BFRs degradation by dehalogenation process. In the aerobic batch test, the dissipation of BFRs was slower as under anaerobic conditions. The order of dissipation rate was as following: HBCD >> TBBPA > BDE-209 ≅ deBDethane. As under anaerobic conditions, deBDethane was practically not removed under aeration (DT_{50} >1000 d). BDE-209 and deBDethane (log P_{ow} >6) are much more hydrophobic than HBCD and TBBPA (log P_{ow} ~6). Therefore, the bioavailability of BDE-209 and deBDethane in aqueous environments will be lower, and this low bioavailability is assumed to limit biologically mediated transformation. Higher dissipation rates for BFRs were not found under anaerobic-aerobic sequential step treatment, particularly for BDE-209 and deBDethane. There was no improvement on dissipation rates of both compounds compared with anaerobic conditions (DT_{50} 35 d for BDE-209 and >1000 d for deBDethane).

In the sludge drying process under UV/Vis irradiation, fast dissipation of BDE-209 and HBCD was observed (DT_{50} 1.5 and 1.7 d, respectively). The order of dissipation rate was as following: BDE-209 ≅ HBCD > TBBPA >> deBDethane. However, after a fast removal in the first 1 to 2 days, a stable phase was achieved, and no further dissipation occurred to the compound. These results showed that dissipation process was highly dependent on the penetration of light into the deeper layer of the sludge. Thus, a continuous mixing of the sludge layer might allow irradiation of all parts of sludge, facilitating more efficient dissipation of BDE-209. In contrast to the rapid dissipation of BDE-209, deBDethane was surprisingly resistant to UV/Vis treatment (DT_{50} >1000 d). The reason for that difference was not clear. For polymer studies, the same behaviour is described for these two compounds in the literature. However, other authors observed a similar degradation of BDE-209 and deBDethane during photodegradation experiments supported by carboxylates under visible lights.

Degradation products of test compounds were detected during batch experiments by GC/MS. Lower brominated congeners (nona- and octa-BDE) were identified during biodegradation of BDE-209 under anaerobic conditions and UV/Vis irradiation.

In total, the current study showed that not all BFRs have to be considered as persistent compounds under different sludge treatment processes. Anaerobic digestion showed the highest potential to degrade different BFRs to trace levels. Anaerobic and aerobic treatment systems were both effective in reducing HBCD and TBBPA concentrations. UV/Vis irradiation effectively reduced BFRs levels from sludge, particularly for BDE-209. DeBDethane was the most persistent compound (DT_{50} >1000) under all test conditions.

7. References

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